

Research Techniques
in use at
The Grassland Research Institute
Hurley

by
Members of the Institute Staff

BULLETIN 45

Commonwealth Bureau of Pastures and Field Crops
Hurley, Berkshire, England



COMMONWEALTH AGRICULTURAL BUREAUX

This up-to-date work describes the experimental techniques in use and the equipment employed at the Grassland Research Institute, Hurley, Berkshire.

They are presented not as being the only, or even the best, techniques for certain types of agricultural experiment, but as techniques tried and found satisfactory. The detailed information given should prove useful to, and save time for, other workers in similar fields: even where circumstances preclude the adoption of the methods and techniques *in toto*, they may serve as a basis for refinements or adaptations.

Price
Forty
Shillings

1871

Research Techniques
in use at
The Grassland Research Institute
Hurley

by
Members of the Institute Staff

BULLETIN 45

Commonwealth Bureau of Pastures and Field Crops
Hurley, Berkshire, England



COMMONWEALTH AGRICULTURAL BUREAUX

*First published in 1961
by the
Commonwealth Agricultural Bureaux,
Farnham Royal, Bucks, England*

*This and other publications of the
Commonwealth Agricultural Bureaux
can be obtained through any major bookseller
or direct from:*

*Commonwealth Agricultural Bureaux
Central Sales Branch, Farnham Royal, Bucks, England*

© Commonwealth Agricultural Bureaux 1961



*Printed in Great Britain by
Lamport Gilbert Printers Ltd.,
Wantage Road, Reading, England*

SUMMARY OF CONTENTS

	<i>Page</i>
FOREWORD	vii
INTRODUCTION	1
PART I	
EXPERIMENTAL DESIGN AND INTERPRETATION	
<i>Chapter</i>	
1 GENERAL PRINCIPLES OF EXPERIMENTAL DESIGN AND INTERPRETATION	5
2 GRASSLAND EXPERIMENTS—PROBLEMS AND PRIN- CIPLES	8
3 THE MAIN TYPES OF EXPERIMENT	12
4 VARIOUS BIOMETRICAL ASPECTS OF EXPERIMENTAL WORK AT THE INSTITUTE	18
PART II	
HERBAGE PLANT INVESTIGATIONS	
5 PLANT PHYSIOLOGY	24
Plant culture	24
Environmental control	26
Measurement of plant growth	30
6 FIELD EXPERIMENTS	33
Examination of new herbage plants	33
Design and management of small-plot experiments	34
Estimation of yield and composition of forage	38
Grass seed crops	47
Botanical analysis in ecological studies	48
7 CLASSIFICATION AND SURVEYING OF GRASSLAND	54
The grassland survey	54

iv TECHNIQUES USED AT THE GRASSLAND RESEARCH INSTITUTE

PART III

ANIMAL INVESTIGATIONS

<i>Chapter</i>		<i>Page</i>
8	EXPERIMENTAL PLANNING AND MANAGEMENT ..	58
9	METHODS OF ARTIFICIAL REARING	66
	Calves	66
	Lambs	68
10	MEASUREMENTS USED TO ASSESS ANIMAL GROWTH	71
11	OTHER MEASUREMENTS	81
	Animal behaviour studies	81
	Parasitological techniques	81
	The measurement of herbage digestibility	86
	Estimation of the herbage intake of grazing animals ..	92

PART IV

PLANT/SOIL STUDIES

12	THE CONDUCT OF LEY AGRONOMY EXPERIMENTS	99
13	ESTIMATION OF WEIGHT OF ROOTS AND UNDER- GROUND ORGANS IN GRASSLAND	101
14	THE MEASUREMENT OF THE EFFECT OF LEYS ON SOILS	103
15	THE MEASUREMENT OF YIELDS OF CEREALS AND KALE AFTER LEYS	110
16	AN APPARATUS FOR SMALL-PLOT IRRIGATION ..	118

PART V

EXTENSION TRIALS

17	PLANNING AND OPERATING EXTENSION TRIALS ..	126
18	EXPERIMENTAL DESIGN	128
19	MEASUREMENTS	131

PART VI

SPECIAL LABORATORY EQUIPMENT AND ITS USE

<i>Chapter</i>		<i>Page</i>
20	DETERMINATION OF THE DRY-MATTER CONTENT OF HERBAGE, FAECES AND SILAGE	135
21	PREPARATION OF DRIED SAMPLES FOR CHEMICAL ANALYSIS	139
22	PREPARATION OF SAMPLES OF FRESH HERBAGE, FAECES AND SILAGE FOR CHEMICAL ANALYSIS	143
23	DETERMINATION OF THE GROSS CALORIFIC VALUE OF HERBAGE, FAECES, URINE AND SILAGE ..	144
24	COLD-STORAGE FACILITIES	150
25	DETERMINATION OF THE CHROMIC-OXIDE CONTENT OF FAECES	153
26	NOTES ON THE DETERMINATION OF TOTAL NITRO- GEN BY THE KJELDAHL METHOD	155
APPENDIX 1	Contributors to the Bulletin	156
APPENDIX 2	List of proprietary products referred to in the text ..	157
APPENDIX 3	Conversion table	161
LITERATURE CITED		162
GLOSSARY		167
PLATES	 facing	168

LIST OF PLATES

<i>Plate</i>		<i>Referred to on page</i>
1	Environmental chamber	29
2	Large pots for growth studies under sward conditions	25
3	Labelling of tillers with label and thread	31
4	Labelling of tillers with pigeon rings	31
5	Wire-netting fence for sheep showing movable iron posts	61
6	A trailer for weighing sheep of all ages	74
7	Rack of buckets for artificial rearing of lambs	69
8	Group feeding of lambs	69
9	Sheep cages for digestibility experiments	87
10	Sheep cage for digestibility experiments	87
11	Detail of sheep harness	87
12	Balling guns used for dosing chromic-oxide capsules	94
13	Combine-harvester showing metal box collecting grain from main auger	112
14	Bagging the grain from the metal tray	112
15	Combine-harvester with canvas sheet collecting straw, cavings and chaff	113
16	Weighing straw from a plot	113
17	An apparatus for small-plot irrigation	118
18	Detail of carriage and spray pipe	122
19	The detachable electric motor for driving the winding drum	122
20	The winding drum and reversing mechanism	122
21	Mechanical post driver for taking soil cores for estimation of weight of roots	101
22	Motor-scythe fitted with an attachment for cutting kale	116
23	Mobile unit used for herbage and botanical sampling	133
24	Trailer for plot cages	133
25	Trailer loaded with plot cages	133
26	Portable weighbridge and trailer	133
27	Portable weighbridge and trailer	133
28	Six-flask Kjeldahl digestion rack	155

FOREWORD

The idea of preparing the present bulletin developed as a result of the numerous enquiries for details of techniques, experimental methods, apparatus and equipment in use at the Grassland Research Institute. These details have been incorporated in this bulletin as a series of chapters written by members of staff. Although we describe techniques and appliances used in our current research programme, we do not suggest that they have attained perfection. Research workers must always seek improvement in techniques and the research team at Hurley is no exception. However, methods employed in our current researches are often the product of long experience, of gradual development, and of consultation with workers elsewhere to whom we tender our thanks. It is hoped that the bulletin will be found useful to a wide range of agronomists and to other agricultural research workers. We also hope that workers elsewhere may be saved the time-absorbing exercise and some of the tedium of developing fresh techniques for measuring the output of grassland and grazing animals.

It should at this point be made clear that only the following classes of stock are used in our experiments:

Cattle. Beef steers, the by-products of the dairy herd, from calf to fat beast.

Sheep. Ewes and lambs, ewe lambs in the year before breeding and, for digestibility trials or as plot grazers, castrated males (tegs).

Work has been done with pigs and poultry but it is now largely discontinued. Dairy cattle are not used.

We are conscious of the shortcomings of many of the methods we use, especially in those field experiments designed to assess precisely the yield of grassland to which a variety of agronomic treatments has been applied.

Important advances have been made during the past decade in grazing experiments, although they had been much neglected in the past, partly, no doubt, because of lack of facilities and the high cost of conducting experiments with grazing animals. Improvement in experimental technique in grazing-animal trials offers a rewarding future; and, with the rapid development of instrumentation, we may look ahead to a time when growth recording in plants and in grazing animals will be a quantitative science rather than a subjective process.

Nonetheless, valuable preliminary work is often possible without full equipment: much pasture work was so accomplished in Britain during the first three decades of the present century. There are even now many instances (in the tropics for example) where lack of equipment should not deter simple experimentation on pasture lands, particularly in areas where there is a serious lack of evidence relating to the yield of grassland subjected to contrasting treatments. Although many of the methods described in this bulletin demand the kind of facilities available at research stations, lack of them should not deter the initiation of an experiment or research

project. Indeed facilities are frequently made available as a result of the preliminary findings of the properly planned field experiment. Grassland agronomists have yet some way to go in the development of precise techniques in animal/pasture agronomy. To be conscious of the shortcomings of existing techniques does not, however, warrant frustration.

Acknowledgements

My colleagues and I are most conscious of the large number of persons and institutions who at various times have given us assistance in connection with development of apparatus or machines which has helped the evolution of techniques in current use. We greatly value these contacts and the free exchange of information which has resulted from them. Whenever possible, sources of information are noted but to do this generally would soon over-burden the text. I wish, however, to express my deepest gratitude to those who have assisted in all manner of ways. It is quite impossible to list each individually and I sincerely hope, therefore, that those who have contributed will know that their assistance is fully appreciated. Members of staff of our sister research institutes and of the Advisory Services have frequently been particularly helpful, and consequently I would like them to know how much that help is appreciated.

I am grateful to the staff of the Commonwealth Bureau of Pastures and Field Crops at Hurley for their assistance and for seeing this Bulletin through the press. I express my gratitude also to my colleagues at this Institute and particularly to the chapter authors, and to my Librarian/Editor, who, together, have brought the Bulletin into being. Everyone in his own sphere has given much thought to its preparation, often at very busy periods of the year and at times when writing and cataloguing had to be done late into the night. The Bulletin represents the combined experiences of the Institute research team of which I am proud to be a member as well as a leader.

WILLIAM DAVIES,

Director, The Grassland Research Institute,

Hurley, Nr. Maidenhead, Berkshire, England

March 1961

INTRODUCTION

The function of the pasture research worker is first to determine the influence of treatment upon crop, soil and animal and to ensure reliable and reproducible results. Second, he should accumulate evidence to explain the various mechanisms that together produce observed results. These are analytical processes, but there is also the further need to integrate the newly acquired knowledge into a practical system designed to replace its existing counterpart. Research has therefore to be based on team work, and different members of the team will have interests in either analysis or synthesis. Some would define the first as fundamental, and the second as operational research. The important point is to ensure that each team is so directed that critical analysis and a subsequent synthesis are provided for within its programme.

This is our approach to research problems. Much of the work must be analytical, whether in the laboratory or in the field. A considerable part of the work in the Animal Nutrition Department, for example, has analytic approach: some researches in Animal Agronomy, and our extra-mural studies, could be classed as operational. These two approaches must be regarded as complementary; and they are essential parts of an integrated research programme.

The requirements of the analytic and synthetic approaches to animal experiments are frequently different. Thus, for example, work of the former type is generally carried out under strictly controlled conditions, usually indoors. This may mean that the number of animals studied has to be limited. In such cases the use of monozygous twins, which have been shown to give an experimental efficiency many times that from using unrelated animals, is desirable.

Operational experiments, on the other hand, are frequently carried out with larger numbers of animals in less controlled conditions, and the limited availability of monozygous twins may impose excessive limitations on the scale of these experiments. We are therefore experimenting with the possible use of "half-sibs" of similar birth, which, although theoretically less efficient experimental animals, should be more useful in such trials.

Whether it be a simple experiment or a complex piece of research, planning is an important (perhaps the most important) phase of the field experiment. This demands much thought by the field agronomist; and it should also be thoroughly discussed with other colleagues, including those with a knowledge of statistical method and experimental design. At Hurley the experimentalist consults closely with the Biometrics Department and together they not only decide the design of the experiment but also define the kind of data to be collected and the manner in which they are best handled biometrically. Having thoroughly discussed the trial with other colleagues during the planning stage, and noted their combined suggestions and comments, the experimentalist himself must be then responsible for the precise design and the ultimate execution of the

experiment. This appears to us the sensible approach to the problem of design and procedure in any experiment. Too often, however, the tendency among field workers (and among those in administrative control) is to demand that the statistician defines and ultimately decides upon the precise design of experiments. The field worker should himself accept full responsibility for both the theoretical and practical execution of his experiments and look to his statistician colleagues for advice rather than for direction. Experimental design provides an invaluable tool in experimental biology, but statistical theory must never be allowed to dominate. Neither should statistics be used by the biologist as an excuse for reducing the number of subjective field observations recorded on the growing crop. Modern grassland research demands the knowledgeable use of biometrics, but it also demands accurate recording, subjective as well as objective, in order to compile a complete picture of sward behaviour all through the phases of growth and development.

In grassland research we frequently need to measure the yield of herbage and its output as measured by live-weight gain or some other character in the grazing animal. It is in animal measurement that difficulties of accurate assessment have proved greatest. Clearly the problem of grassland recording becomes more complex when the grazing animal is introduced. The quantity and the quality of the herbage offered to the animal has a profound effect upon it. It is less obvious but equally true that the animal, in turn, has an important influence upon the amount, the quality and the continuity of the food supply from the swards upon which it grazes. The animal may make or mar its own pasture. Good pasture/animal management maintains the sward at a high level of productivity, whereas indifferent management may quickly suppress valuable plant species and depress yield.

At Hurley, all herbage cuts are reduced to a dry-matter basis; and this is desirable in every situation where accurate comparisons between treatments are to be made. This procedure, however, demands adequate oven-drying facilities not available at every centre. However, those with inadequate facilities need not be deterred from initiating experiments and recording green weights and air-dried weights of herbage cuts, provided the limitations of such data are kept in mind.

In the majority of our herbage studies we determine, in addition to dry matter percentage, the nitrogen content of the herbage and, in particular experiments, the contents of the major minerals* and of certain trace elements*. In specialized experiments the content of amino-acids* may be determined or a detailed analysis of the nitrogen* and the carbohydrate* fractions made. In yet other trials herbage digestibility may be tested using sheep and cattle. *In vitro* (artificial rumen)* digestibility may also be recorded. Energy values are determined by the use of the automatic adiabatic bomb calorimeter, an instrument developed in the Institute workshop.

In experiments with grazing animals at Hurley the performance of beef cattle, and that of sheep, is studied under different regimes of live-

*These techniques are not described in this bulletin, as they are more suited to specialized chemical publications.

stock management and grassland treatment. In such experiments the animal is used as a recording instrument. The range of the recordings will depend on the purpose of the experiment concerned. In most situations, however, the number of grazing days and the pattern of live-weight gain in the beef animal and in the fattening lamb are recorded. The data may be calculated in terms of live-weight gain per acre, per animal per day, and per animal over the grazing season.

In certain animal experiments additional data, e.g. the dead weight at slaughter or the commercial grading and cash value received for the carcasses, are recorded. In other trials a more detailed analysis is made of certain joints and a record made of the weight of major parts of the carcass after being jointed by a commercial butcher. Other experiments may involve detailed recording of growth rates of different parts of the animal, e.g. comparative bone development, rumen development, girth measurement, etc. Some of these procedures involve the slaughtering of immature animals.

In animal experiments designed to make a comparative evaluation of pasture types or of pasture treatments, it is necessary, before beginning field work, to decide precisely what product is to be grown and what evaluations are to be made. The end-product must be common to all treatments. This decision is important because the type of pasture and the treatments superimposed upon it within an experiment should be conditioned by the product desired. Thus experiments in which the output of fat lamb per acre provides the yardstick of herbage output are likely to need a range of treatments different from that of experiments dealing with adult fattening bullocks.

A serious shortcoming in experiments with grazing animals is the lack of a 'standard' animal which can be used in the way a mechanical recording instrument is employed. The grazing animal is not only subject to the usual variations inherent in any biological unit, but also it often carries parasite burdens which may at any time reach clinical proportions and cause differences between individual animals within an experimental group or between groups. This has happened frequently in our trials. Internal parasites in sheep, for example, are almost universal; but the level of infestation may only be sub-clinical, in which case the appearance of the animal may not suggest that it is diseased. Commonly, however, the influence of internal parasites of sheep is apparent; in lambs the infection often results in poor development or death. Similar observations apply to cattle, in which parasitic bronchitis (husk) is a common ailment.

Unthriftiness may create further lack of uniformity in experimental groups of animals, and, where their output is being measured in comparative tests (as when comparisons of various herbages are made), extreme care must be exercised in the interpretation of the experimental data. This matter has been of some concern in the Hurley research programme, and animals must be kept reasonably free from parasites. The methods we have used to provide an environment in which sheep experiments can be carried out free from any significant interference from internal parasites are fully described in Part III, Chapter 8, pp. 63-4.

While this situation can be maintained, it provides a valuable tool for assessing grassland treatment and its influence on animal productivity.

Since the normal sale products of grassland are milk, wool, meat and hides, the pasture research worker must recognize that herbage studies (with little or no animal influence) and grazing-animal studies are complementary. It is difficult to place an economic value on herbage as an end-product, because there is only a limited market for grass (and this is largely in terms of hay). Consequently, although herbage agronomists should assess the comparative yield of grass resulting from differences based on, say, seeds mixtures, varieties, varieties $\times$ treatments and natural or sown grassland, it is unwise to translate such data for use by the farmer until they have been checked by experiments involving yields in terms of animal product. While the herbage agronomist can indicate differences, e.g., that variety 'A' is more persistent, high tillering, leafy and productive than variety 'B', it is for the animal agronomist to show whether these differences can be translated into yield of a defined animal product when those varieties are established as part of a mixed sward containing volunteer as well as sown species. Few of the trials yet reported upon in Britain or elsewhere have in fact been able to detect with any precision small sward differences in terms of yield of animal product.

The methods described in this bulletin and the appliances used (often derived from trade sources and modified in our workshop, but sometimes built here), are the result of trial and error over a period of two decades. We have not achieved the ideal but a stage has been reached at which the techniques now used serve their purpose and make it possible to assess with reasonable precision the broad characteristics of herbage swards, the reaction of the animal to them, and the influence of the pasture/ grazing animal complex upon the crop potential, and on other characteristics of the underlying soil.

PART I

EXPERIMENTAL DESIGN AND INTERPRETATION

In this Part an attempt is made to give a general picture of the biometrical techniques used at Hurley, with particular reference to the statistical problems which are peculiar to grassland experiments. It will become apparent that some of the biometrical techniques in use have severe limitations and that considerable research is needed.

We can conveniently arrange grassland experiments into three main classes according to the methods of defoliation and assessment used:

- (1) Cutting only (no animals present),
- (2) Cutting with animals present only to impose treatments (not as measuring instruments),
- (3) Animals as the major measuring instrument.

This order also usually corresponds with increasing difficulty of execution and interpretation. Experiments in classes (1) and (2) can be carried out on small plots whilst experiments of type (3) usually require extensive facilities in land, animals and labour.

In this Part some general principles of experimental design and interpretation are briefly mentioned. Next, problems particularly associated with grassland experiments are discussed and some suggestions put forward. Pot experiments and the three main types of field experiment are then discussed, and lastly various miscellaneous biometrical aspects of the work of the Institute are considered.

CHAPTER 1

GENERAL PRINCIPLES OF EXPERIMENTAL DESIGN AND INTERPRETATION

In this chapter we mention some of the accepted principles applicable to the design of experiments in general. Much of this will be found in standard textbooks on experimental design (e.g. Cochran & Cox, 1957) and is restated below merely for convenience and completeness.

Define the problem and hence the objectives of the experiment as clearly as possible

Experiments are normally carried out to provide answers to questions. If a vague question is asked, it is unlikely that a precise answer will be received. It is always a good plan to draw up a schedule, stating the

objectives of the experiment, treatments proposed, measurements to be taken, type of design, range of conditions over which the conclusions are to be applied, skeleton analysis of variance, etc., before the experiment is laid down.

Avoid unnecessary modifications once the experiment has started

Modifications may be enforced by unforeseen practical considerations or they may derive from an idea suggested either by the results of the experiment to date or by knowledge acquired after the experiment has started. In any case, the consequences should be considered very carefully before making the modifications, for, if various comparisons are thus rendered invalid, the net result is a loss of information.

Treat unexpected results with caution

On examining the data, effects are often suggested which were not specified as part of the objectives when the experiment was designed. These effects cannot be regarded with the same degree of confidence as those which the experiment was designed to estimate or test. The same experiment cannot be used both to construct and to test an hypothesis. It should be remembered that careful examination can detect a pattern in any finite set of data, and, in general, the smaller the set of data, the easier it is to construct a meaningful explanation of a pattern. Hence, effects suggested by the data must be treated with caution and should normally be tested in future experiments. In other words, whilst data must be examined very critically there is a risk of reading too much into them and of accepting conclusions which require independent confirmation.

Bear in mind the implicit assumptions

Underlying any experimental design and its associated analysis is a number of mathematical assumptions, e.g. one usually assumes additivity of block and treatment effects, homogeneity and normal distribution of errors, etc. If these assumptions are seriously violated, the conclusions drawn from the analysis may well be invalid and misleading. Unless one takes the trouble either to understand the assumptions and their implications or to get professional advice, there is a real risk of running into difficulties by choosing a design and analysis because they have been successfully applied to a problem which is superficially similar to the one being studied.

Randomize and replicate

The importance of randomization in avoiding bias due to extraneous variation, and in providing valid estimates and tests of significance, is well known. Adequate replication is also most important. It is often possible to assess whether the proposed degree of replication is adequate, for, if one knows from previous experience the approximate magnitude of the experimental errors, it is easy to determine approximately the probability of detecting differences of a given magnitude (tables are given in Cochran & Cox, 1957, pp. 20-8). If the resources available are such

that there is little chance of detecting differences of the order in which one is interested, then there seems to be little point in proceeding with such an experiment unless it is one of a series all of which will ultimately be considered together.

Remember that estimation is usually more important than significance

There are two main ways of looking at an experiment. Suppose we take a very simple experiment comparing two treatments A and B. An analysis of variance shows a significant difference between A and B. To state that A is significantly greater than B is not particularly informative since agricultural significance and statistical significance are by no means identical. A difference of 100 lb/acre might be highly significant (statistically) yet of no agricultural importance, whilst on another occasion one of 1000 lb/acre might be non-significant statistically yet if it were real it would be of considerable agricultural importance. Absence of statistical significance may be due to the fact that the underlying effect of the treatment is negligible or it may be due to bad or inadequate experimentation. Therefore, whether or not statements of significance are made, it is highly desirable that the errors of estimation associated with the data should be quoted, i.e. tables of means and standard errors are almost always preferable to statements of significance. The quoting of "least significant differences" is not recommended since it is not in general true that *any* difference which exceeds the least significant difference can be regarded as significant.

The main points of this chapter may be summed up as follows: spend as much time and thought as possible on the planning of the experiment for it is false economy to do otherwise; put down on paper the objectives of the experiment, the means by which these will be achieved and the skeleton analysis of variance; bear in mind the implicit assumptions, and, if in doubt about the proposed design, consult a statistician with experience in your particular field before you start the experiment.

CHAPTER 2

GRASSLAND EXPERIMENTS—PROBLEMS AND PRINCIPLES

Lack of a single end-point

The type of agricultural experiment (e.g. with cereal crops) for which standard experimental design theory was developed normally has a clearly defined end-point. The experiment can therefore be designed to estimate efficiently the effect of the factors under investigation at this end-point (e.g. yield of grain at harvest). However, in many grassland experiments one normally has a series of harvests in each season, all of which may be of equal interest. There is the additional complication that each of these harvests itself affects the subsequent ones, since regrowth will depend, among other things, on the stage of growth and height of defoliation. Treatments will often alter the composition of the sward differentially; such effects may or may not be cumulative.

At present we do not know what is the best way to deal with such data. Because of the correlations which exist between successive harvests, an analysis of variance which includes harvests as a simple factor may be very misleading. Similarly, the inclusion of harvests as "split-plots" is unsatisfactory. However, total yield over the season is not particularly informative, so one tends to analyse each harvest individually, but to bear in mind when interpreting these analyses that harvests influence each other and that all harvests are taking place on the same plots. As a consequence of the last point, some peculiarity of the particular physical lay-out employed may well be reflected in every harvest.

Here, then, there is a need for a technique which will view the process as a whole. This problem is being investigated at this Institute.

Multiple measurements

A problem which has much in common with that of successive harvests on the same plots is the general one of multiple measurements. For example, one will often measure green weight, dry-matter percentage, crude-protein percentage and botanical composition for every plot and from these further measures can be derived. Once again, one is faced with problems of interrelationships and the peculiarities of the particular field lay-out. Normally, we attempt to designate one or two of these measures as of major importance and analyse them alone, regarding the others as additional information which assists in explaining the results on the main measurements. Sometimes analyses of covariance are carried out in order to obtain more information on interrelationships.

However, general interest in the theory and applications of multivariate analysis is becoming much greater now that electronic computers are available to undertake the very heavy computing which is almost invariably involved. We may therefore hope for rapid advances in such techniques.

The general pattern of variability

The pattern of variability in the grass sward is usually very complex. Reasons for this include: (a) the sward is seldom a pure stand; (b) the composition of the sward is usually in a state of continual change; (c) grasses and legumes have very complicated growth habits.

We frequently find that, at Hurley, variation between adjacent areas approaches the same order of magnitude as that between areas which are some distance apart. As a result of this the use of designs which require small compact blocks as opposed to those (usually simpler) designs which permit quite large ones is not necessarily advantageous. In fact, as will be mentioned later, the disadvantages may well outweigh the advantages.

Unequal variances

Some types of grassland experiment suffer from the complication of unequal error variances for different treatments. This can sometimes be dealt with by the use of an appropriate transformation of the data before analysis; the logarithmic transformation has been found to be the most useful one. Transformations are usually of value where the mean and the variance are related in some fairly simple way. However, in some experiments situations can occur (e.g. in parasitological work) where the effect of a particular treatment is to alter drastically the variance without greatly affecting the mean. It is then important to ensure that the design adopted enables one to deal with such a situation and from this point of view the simpler designs are much more satisfactory.

Choice of experimental units

In some experiments there may be doubt as to what should be regarded as the basic experimental unit for the purposes of the analysis. An example where there is normally little doubt is the cutting type of experiment when more than one sample per plot is taken. Here the unit when assessing treatment effects is the plot and not the individual sample, though information on individual samples is useful in deciding whether the sampling is adequate. The situation is often much less clear cut than this in animal experiments in which groups of animals are subjected to treatments and also in some pot experiments where more than one plant is grown in a pot. Here both competitive and co-operative effects between individuals in a group may be important. It is perhaps worth remarking that if a small group of 2 or 3 animals or plants is the unit, considerable difficulties may ensue if one or more individuals within a group are lost during the course of the experiment because of death or some other mishap. One therefore normally aims for each group to include 1 only or else at least 5 individuals. Some aspects of this problem will be discussed in Chapter 3.

The effects of climate and the need for replication in time

Any attempt to draw conclusions that are based on only a single year's experimental data is dangerous because, apart from anything else, climatic conditions vary so much from year to year. An experiment which continues for several years by repeating the same treatments on the same plots every

year still suffers, to a large extent, from this disadvantage since conditions in, say, the sowing year may greatly influence the whole of the subsequent course of the experiment. Some form of true replication in time, as well as in space, is therefore highly desirable and is often provided by extra-mural experiments (see Part V).

Practical limitations and experimental mishaps

It would be unrealistic to ignore the limitations which practical considerations sometimes place on experimental design and conduct. It is pointless to carry out an experiment which gives formally valid estimates and errors and yet in fact does not really answer the original question, because, e.g. the treatments have been modified too much in order to make the experiment conform to a standard lay-out. In such cases it is incorrect to call the experiment statistically sound.

There will inevitably be situations in which it is not possible to follow the most desirable lay-out because of physical limitations imposed by the field techniques under study. For example, to give sensible results the size of plots used for grazing must not drop below a minimum for a particular type of experiment, therefore one will often use grazing as main-plot treatments and fertilizers as split-plot treatments in situations where it would be theoretically desirable to reverse the roles. Similar limitations are often imposed by farm operations such as ploughing and drilling. It is usually possible for the statistician and the experimentalist to reach a reasonable compromise by careful discussion.

It must be emphasized that any sacrifice of the usual requirements of design theory will inevitably make interpretation of the data more difficult and less certain; and it should not be undertaken lightly. One unsatisfactory solution of such situations is sometimes apparent in the literature. It is reported that data were not amenable to statistical analysis; and yet conclusions are drawn as if the data had been analysed (usually as if the degree of precision were very high).

In field experiments, particularly those with animals, there is always a risk that data will not be available from one or more of the experimental units when the time comes to analyse the data. This may be due to illness or death of the individual unit, loss of records, etc. It is therefore wise to ensure that the design used is such that allowance for this can be made without too much difficulty; this is particularly desirable in experiments which continue for a long time and in those which involve a large number of analyses. Even so, caution must be exercised in using the standard missing-plot and covariance adjustments, for, if the loss of information is in any way a reflection of the particular treatment imposed, these adjustments may lead to biased estimates.

Conclusions

Some general conclusions on the design of grassland experiments may be drawn from the foregoing and from the experience gained at this Institute.

At present, we use the standard types of experimental design and separate analyses are usually made for each variable and for each period of time in which one is interested. In interpreting these analyses the presence of interrelationships must be borne in mind, and, in particular, the limitations imposed by the fact that the same plots are being used all through the experiment.

Simplicity of design

We feel that simplicity of design is a major consideration; and randomized (complete) block designs are probably the most widely used at Hurley. Split-plot designs, although not always particularly desirable in theory, are often necessitated by practical considerations. However, the fact that two separate errors are involved is often inconvenient and can lead to a serious loss of precision in small experiments.

Randomized block designs, by comparison with incomplete-block designs, include among their advantages the following:

- (a) In general, they are easier to conduct and the analysis of results is easier to compute. This latter may be of some importance if there are a number of sets of measurements, made at different times and perhaps on more than one variable at each time, each to be analysed separately. Also, one normally need make no adjustment to treatment means whereas in some complex designs the means must be adjusted for blocks before they can be properly examined.
- (b) Mishaps leading to missing data can be dealt with relatively easily. In extreme cases, removal of a complete treatment or a complete block does not alter the pattern of the analysis though it may, of course, dangerously reduce the degrees of freedom for the estimation of error.
- (c) Following (b), it is relatively easy to make allowance for treatments which have different error variances (when transformation is not a satisfactory solution) since groups of treatments can be isolated.

Randomized blocks are, of course, normally less efficient (in the sense of the magnitude of the error variance) than the complex designs, but under our conditions this loss of efficiency does not seem to be great and it is probably more than compensated by the advantages listed.

Replication in time

For reasons stated earlier, replication in time as well as in space is highly desirable. If one is prepared to lay down (say) 6 replicates, then it would be much better to establish 2 in each of 3 successive years, than to lay down all 6 in one year.

CHAPTER 3

THE MAIN TYPES OF EXPERIMENT

In this chapter we deal with pot trials and with the three main types of grassland experiment. We shall not, however, consider the 'test-crop' phase of soil experiments here: their physical lay-out largely depends on the requirements of the ley phase, the principles of experimental design for cereal crops are well known, and the relevant practical points are dealt with in Chapter 15.

Pot experiments

Before we examine the main types of field experiment, some mention must be made of pot experiments, which are normally carried out in the glasshouse. The physical lay-out of such experiments is made more flexible by the ease with which it is possible to vary the positions of pots. However, there are usually more treatments in these experiments than in field ones; and there may be marked positional effects in the glasshouse. Two ways of dealing with this situation are: (a) the use of skilfully designed blocks (which will often require incomplete-block designs); (b) regular re-randomization of the positions of the pots. The advantages and disadvantages of (a) are well known but little is known about (b) although the principle has been discussed by Kempthorne (1957). The disadvantage of (b) is the necessity for the regular shifting of the pots, but this can be made part of the routine—for instance when the plants are watered. An advantage of (b) is that it eliminates the complex calculations which sometimes result from the use of incomplete-block designs. Such calculations can be very tedious if a large number of analyses has to be made. Also 'missing' pots caused by plants dying, etc. are more easily dealt with when method (b) has been used.

When carrying out pot experiments, one often wishes to remove small samples at regular intervals during the course of the experiment in order to obtain a semi-continuous record, which is of considerable value when interpreting the final results. This can be done in a number of ways but it is wise to make detailed provision for it before the experiment is started. If the lay-out comprises a number of replicates in complete randomized blocks, one or more blocks may be removed at random at each sampling date. Alternatively, if regular re-randomization of all pots is carried out, the sample pots could be selected entirely at random. Again, if very large blocks are acceptable (positional effects being assumed to be negligible) a single replicate may contain $a + b$ pots of each treatment—one is removed at each of a sampling occasions and b remain for the final, more detailed, assessment. Sometimes it is necessary to replace the sample pots by 'dummy' pots in order that the micro-environment of the remaining pots may be disturbed as little as possible.

One very important decision which has to be made when designing pot experiments is the number of plants per pot. Unless one is studying germination, for example, it is wise to have an equal number in each pot and to avoid very small numbers (excepting one per pot) because of the difficulties which arise if one or more plants in a pot dies or in some other way becomes so atypical that it has to be excluded from the results. The disadvantage of having a single plant in each pot is the very high variability of individuals. Against this, the more plants there are in a pot, the earlier in their life is competition likely to affect them.

If there is only one plant in each pot, standard missing-plot techniques can be used in the event of plants dying, provided that one can assume death is in no way connected with the particular treatment applied. This important assumption is, of course, implicit in the use of missing-plot techniques. If it does not hold, the application of the missing-plot technique will lead to biased estimates of the treatment effects.

Suppose now that there are 10 plants per pot and that some plants die in one or more pots. Again we assume that death is not connected with the particular treatment(s) involved. Let us, for example, consider dry-matter yields. We can now use either (a) mean yield per plant per pot based on the actual number of surviving plants or (b) total yield from each pot (this is, of course, for the purposes of this discussion equivalent to mean yield per plant per pot based on the number of plants originally present). In practice, unless there are a large number of deaths, either measure will usually give satisfactory results; (b) is probably a little safer if competition is operating or if it is at all likely that death is not independent of the treatment applied. If there are several deaths in the experiment, an analysis of covariance of the yield of each pot on number of surviving plants in each may give some indication as to which method is the more suitable.

Experiments excluding animals

These are normally carried out on small plots (e.g. 6×2 yd) and are used to compare fertilizers, sowing rates, heights and dates of cutting, species, varieties, herbicides, etc. They often represent the early 'screening' stage of an investigation, because it is practicable to test simultaneously far more factors than one can hope to test in full scale animal experiments. Any attempt to extrapolate from small-plot experiments to grazing conditions requires considerable skill and caution (in the same way extrapolation from spaced-plant trials to sward conditions can be very hazardous). Sometimes, of course, as in some herbicide trials, these experiments can provide all the evidence required, but usually the more promising treatments will later be tested under grazing conditions (see the following two sections).

Assessment will usually be by yield and composition of herbage cuts (by autoscythe or mower), but quadrat techniques and estimates by eye are sometimes used.

When herbage cuts are to be taken the size of plot used will usually depend on the sampling tool to be used. Frequently about 50% of the plot

is sampled. Any attempt to sample a much higher percentage than this is liable to run into difficulties because of edge effects.

Coefficients of variation of dry-matter yields from these experiments fall in a wide range but, in general, to stand a reasonable chance of detecting a true difference of 10% or less between treatments, considerable care must be exercised in the experimental techniques. Since these experiments are relatively straightforward to carry out, standardization of technique is not very difficult and is well worth the effort. For example, direction of cut (see p. 35) is an important factor. Again, if long delays are likely to occur between sub-sampling in the field and the determination of dry-matter percentage in the laboratory, the polythene-bag method (p. 132) is preferable to the chip-basket method (p. 44). In most circumstances, the use of chip-baskets will lead to consistently higher estimates of dry matter than will that of polythene bags but this is not necessarily a serious criticism since, as is pointed out in Chapter 20, the definition of dry matter is arbitrary.

Lastly, in this type of experiment (except perhaps in the very early stages of an investigation) it is reasonable to expect that there will be adequate replication and correct randomization. It should also be practicable to establish two or more replicates in each of two or more years.

Experiments with animals present (as part of the treatments)

Here the main assessment is still made on the herbage; any animal measurements are supplementary to it.

Larger plots are normally required for grazing and this suggests the use of main plots for grazing and split plots for the treatment types mentioned in the previous section. Alternatively, plot size may sometimes be kept down by fencing to keep the stock out of certain plots rather than in others (see p. 35).

The risk of edge effects in grazing plots must be kept in mind (see, for example, p. 35). Normally, the proportion of a grazing plot which is sampled by herbage-cutting techniques will, of necessity, be very much less than is usually the case in the previous type of experiment.

One expects field variation to be somewhat higher in this type since additional unevenness may be introduced by dunging, urinating, trampling and selective grazing. To minimize these effects, it is usually desirable to top over plots with a mower after they have been grazed.

When a number of plots receive common grazing, as usually occurs in these experiments, two problems arise. First, there is the risk of fertility transfer; and second, there is the possibility of differential grazing (which is likely to be closely related to the density of stocking). These points are discussed on p. 37.

An interesting combination of this and of the preceding type of experiment is the technique of alternate mowing and grazing described on p. 37. This will reduce differential effects from both grazing and dunging but will usually require more plots than an equivalent simple experiment of either type.

Animal experiments

This includes essentially different classes of experiment ranging from the digestibility trial lasting a few days to the large-scale husbandry experiment lasting several years. The various aspects that need to be considered when designing animal experiments are so numerous that we can hope to touch on only a few of the more important ones here.

In the previous chapter, the experiments were concerned with the influence of the animal on the pasture. In animal experiments we are concerned firstly (usually in very short-term trials) with the effect of the herbage on the animal and secondly, more often, with the interaction of the herbage and animal (to avoid complication we do not bring the soil factor explicitly into the discussion). In this context, the importance of adequate herbage data to assist in interpreting the animal data is obvious.

Because of the interaction of sward and animal, it is not generally possible to predict the outcome of a proposed long-term experiment by regarding it as a series of independent simple short-term trials. This interaction also raises considerable problems in the management of long-term experiments, since at certain critical periods of the experiment, faulty management decisions may drastically alter its succeeding course. Before the experiment is started therefore the experimental objectives and the details of the managements to be used must be clearly defined. There is a subjective element in management (e.g. decisions on stocking rate and density, when to move stock) which is not present to such a marked extent in the other types of experiment. As we achieve a greater understanding of the principles of stock management, the basis of animal experiments will become more objective. For some time to come, however, it seems that personal judgement will be an important factor and that every care should be taken to define procedures in advance in such a way that the amount of freedom left for on-the-spot decisions is kept to a minimum.

In many experiments, live-weight increase is the obvious measure of the success of a treatment. Unfortunately, being formed as the difference between two live-weight measurements, both of which are subject to considerable errors, live-weight increase is so variable that it is usually quite unsatisfactory for short-term trials. Various methods have been tried to reduce this extraneous variation, e.g. covariance on initial weight, careful allocation of animals to treatments, use of closely related animals, fasting to reduce the differential effects of fill (see p. 73). At present, however, one must in general accept the need for large numbers of animals if the results from live-weight increase are to be reasonably precise.

There are two questions in animal experiments which are usually of key importance and are interrelated: (a) Do we regard the group or the individual animal as the experimental unit?, (b) What is the appropriate size for the group?

The decision in case (a) is by no means an easy one. Practical convenience usually suggests the use of the individual rather than the group, but the use of the former is often unjustifiable. In regarding the individual

as the unit (and hence the replicate) one is assuming independence of the animals in relation to the measurements being studied but, if they are grazing together, this assumption may well be unreasonable. Independence can break down in two main ways: by competition and by co-operation between the members of a group. Competition will occur, for example, when a limited amount of feed is available so that if one animal eats more, others must eat less. Co-operation may occur if the group tends to graze as one for set periods of time (herd effects).

We may distinguish three basic kinds of design depending on the assumptions one is prepared to make:

- (i) There is one area of land for each treatment. A group of animals is on each area, and individuals are regarded as replicates.
- (ii) The areas of land are replicated (in randomized blocks, say) but the same group of animals grazes in turn all the replicates of a treatment. Individuals are again regarded as replicates.
- (iii) Areas are as in (ii), but a separate (usually small) group of animals is on each replicate area.

Type (i) is the simplest to carry out but makes the most assumptions since, in addition to ignoring group effects, it ignores random physical differences between the areas of land. Type (ii) to a large extent removes this latter objection, but retains the assumption of animal independence whilst Type (iii) overcomes this.

However, one is still up against problem (b). First, one has the choice of stocking rate (e.g. should it be fixed or variable, high or low) but even when this has been decided upon, there still remains the size of group (and hence the area of land needed). It is by no means certain that one will get the same pattern of results from an experiment with 2 animals per plot on 1-acre plots as from another, otherwise identical, experiment of 10 animals per plot on 5-acre plots, although the stocking rates are identical. This problem is closely related to the first one since co-operative herd effects, for example, are likely to depend upon the size of the group.

It is also important to make the obvious distinction between density of stocking and stocking rate—the former being the relation of number of stock to the area actually being grazed whilst stocking rate is usually the relation of number of stock to the total treatment area over the period of the experiment. Thus with strip-grazing, for example, one may have very high densities of stocking yet only a moderate stocking rate.

The design of experiments with sheep is normally easier than those with cattle in the sense that stocking rates are much higher. It is therefore quite practicable to carry out fully replicated experiments with a group of 4–6 sheep in each replicate plot of a treatment—this has been done a number of times at Hurley. Similar experiments with cattle require much more extensive facilities but they have been attempted at Hurley (see p. 59).

Even if one is prepared to consider individual animals as replicates, if only one area of land per treatment is used (as in (i) above), it is being assumed that chance variations between the treatment areas (e.g. soil, sowing rate, exact composition of sward) are irrelevant to the treatment comparison; i.e. that the treatment effects are independent of the factors

that make two identically treated areas of land give different yields. If one is concerned in assessing different swards using the animal as a rather complex measuring tool, then the case for replication and randomization is similar to that for the small-plot experiments described earlier. Clearly, one can draw conclusions from unreplicated experiments but in order to do so a number of strong, usually unverifiable assumptions have to be made: one should always aim to reduce their number and strength.

In many animal experiments, however, replication in time is even more important than spatial and group replication within any one year. For example, the weather may drastically affect the worm burden in sheep and thus lead to widely differing results in a wet as opposed to in a dry year.

If circumstances force the scale of animal experiments to be inadequate, then it is doubly important that any one experiment should be repeated a number of times (normally with fresh land and animals).

Allocation of animals to groups is discussed in Chapter 8 but it is worth noting that the best method of allocation for replicated groups may not be the right one for groups where the individual is the replicate. In either case, subjective balancing of groups (e.g. to ensure that all treatment groups start with identical mean weights) is most undesirable. Inequality of initial weight can be allowed for by covariance analysis. We frequently find, however, that in an experiment using a single class of animal, live-weight gain is virtually independent of initial live weight provided that there are no extremely heavy or light animals in the experiment.

The problem of sick animals is sometimes present. Provided sickness can be assumed to be independent of treatment differences, procedures similar to those given when plants in pot experiments die may be applied. If, as is usually the case, stocking rate is liable to affect the results, then an animal with a previous history as similar as possible to that of the sick one should replace it, but in most cases only the remaining animals of the original group will be measured to contribute to the final results.

CHAPTER 4

VARIOUS BIOMETRICAL ASPECTS OF EXPERIMENTAL WORK AT THE INSTITUTE

Botanical estimates by quadrat- and scoring-techniques

These methods are used occasionally on their own and frequently in conjunction with yield data. They include simple visual estimates of quality and area cover, tiller counts, and point-quadrat estimates of various types: most are discussed in detail on pp. 48–53.

Estimates by eye include simple scoring systems. Scales are arbitrary and the number of categories in the scale is often restricted to 5. It has been suggested that it is better to score from 5 to 9 than from 1 to 5. The scale is usually calibrated in relation to the particular experiment. Probably the most useful method is to walk over the experimental area several times to get an idea of the average value, call it 7 (if using the scale 5–9) and assess all plots in relation to it. The discrete units of the scale are subjectively chosen to correspond with visible differences. An alternative, less satisfactory, method is to examine the experimental area, rate the worst plot 5 and the best plot 9 and then estimate all others in relation to these extremes. This has two disadvantages—it places undue emphasis on the extremes and it forces one to have five discrete values although it may, for instance, be possible to distinguish only three (in such a case scores by the first method would all fall in the range 6–8).

In spite of the subjective nature of scoring, it is possible to get surprisingly good agreement between observers if procedure is standardized at the start. Subjective methods such as these may be used to collect a semi-continuous record of what is happening throughout the course of an experiment—an invaluable aid in the interpretation of final yield data.

Square quadrats (usually with 6-in. sides) are often used for the visual estimation of percentage cover of a species present and for tiller counts. Quadrats may be located at random at every sampling, but, in assessments concerned with change rather than absolute value, it may be useful to have permanently sited quadrats. Methods for locating quadrats are discussed in the section on field sampling below.

Point-quadrat techniques are mainly used to describe the botanical composition of swards. However, a technique for estimating a "height index" has been developed (Spedding & Large, 1957); and recently a modified point-quadrat method has been proposed for the estimation of leaf area index in the field (Warren Wilson, 1959).

In measuring change from year to year, it is necessary to ensure that the measurements are taken at comparable times, otherwise there is a risk of ascribing to treatments effects mainly due to differential growth habits.

The analysis of data produced by the above techniques occasionally demands their transformation. In general, such transformations are

empirical and it is usually only necessary to transform when the data cover a very wide range.

Indirect methods of estimation

Under given experimental conditions, it is frequently impracticable, and sometimes even impossible, to measure directly a variable in which one is interested. A search may then be made for a closely correlated variable that is more easily measurable. However, although this method is widely used, it involves an assumption which may be false. The assumption is that the relationship between the variables is constant over the whole range of treatments under examination. When relationships, determined under a very limited range of conditions, are applied to much more extreme conditions, the results may be most misleading.

Two contrasting examples are provided by techniques described in this Bulletin. In, for instance, the estimation of leaf area from linear measurements of the leaf, the relationship is very close and available evidence suggests that it remains constant. On the other hand, the relationship involved in estimating herbage digestibility from faecal nitrogen is not nearly so close and there is ample evidence that it is markedly affected by a number of factors which may well vary between treatments in an experiment (e.g. species, date of cut).

One should therefore check the validity of any technique of this type whenever it is used under new conditions; also, unless there are strong grounds for believing in its constancy, it is worthwhile occasionally to check that it still holds under roughly the same conditions as those for which it was originally established—this is particularly important if the relationship is essentially empirical.

Field sampling

Sampling, like many other aspects of experimentation, usually represents a compromise between the practicable and the desirable. In small-plot work, a single sample is often 50% of the plot. To sample a much greater proportion would, in fact, usually be undesirable because of the existence of edge effects; they are particularly important where extreme treatments are adjacent, especially in weed- or insect-control experiments. They are also very important in experiments in which animals are present.

Where a number of samples is to be taken from each plot (and where the area sampled is small in relation to the area of the plot) various methods for selecting sample sites are available. The choice is usually between purely random, stratified random (the area is divided into a number of sections, one or more samples being selected at random from each stratum), and systematic (e.g. starting from a given point, a sample is taken at regular intervals along a line across the plot). There are advantages and disadvantages to all these methods but, if carried out correctly, they share the virtue of objectivity. Subjective attempts to obtain a random or a representative sample can produce very biased results. Such 'random' methods as throwing a stick and taking a sample where it lands still contain a considerable element of subjectivity and are not, in general, to be recommended. A good way of selecting a sample site is to allocate to each sample

a pair of numbers (from a table of random numbers) and to use these as co-ordinates. They need not be co-ordinates relative to the corner of the plot, but may be measured from the site of the previous sample. The whole procedure is thus completely determined before the person taking samples sets foot in the plot.

Throwing a quadrat and sampling the area where it lands can lead to biases which tend to become more serious as quadrat size decreases. For instance, bias due to a 3-in. quadrat bouncing away from clumps of herbage may be quite serious. This may to some extent be overcome by incorporating the quadrat in the centre of a very much larger frame (say with a 12-in. side). Sampling methods dependent upon random co-ordinates (either over the plot as a whole or within strata) are preferable, although perhaps more time-consuming.

Permanent quadrats are usually sited systematically, e.g. across a plot at regular intervals, their sites being marked for repeated identification by two corner pegs knocked down to ground level. Their approximate positions are determined by means of a marked rope across the plot: the pegs are then usually easy to find. Good coverage of the plot is particularly important when using permanent quadrats and so several rows of quadrats would normally be included in each plot.

The number of field samples needed depends on: the pattern of variability in the sward (including the relative values of within- and between-plot variation), the type of sampling instrument, the purpose for which the data are required and on many other factors. In the early stages of any investigation it is always useful to take more than one sample at random per plot and to keep separate either individual samples or at least two groups of equal size for each plot in order to estimate sampling error and plot error. Consideration of the relative values of these errors will indicate whether a stated increase in the sampling intensity is likely to lead to a marked reduction in the errors associated with the final estimates of treatment effects. Problems of this kind will be dealt with in the next section.

It is characteristic of the types of measurement made in experiments at this Institute that the coefficient of variation tends to decrease slowly as the mean value of the measure increases, although absolute error tends to increase. One contributing factor is errors of measurement, which tend to remain fairly constant over wide ranges and thus exert proportionately less influence at the higher end of the range. For example, suppose that a field balance reading is accurate to 0.2 lb then the errors introduced by the balance will be relatively less important when weighing a sample of 25 lb than when weighing one of 5 lb.

It is also worth noting that too much emphasis can be placed on the coefficient of variation—it has meaning only in relation to the magnitude of the differences concerned. Selection of measures solely because they have low coefficients of variation is not, therefore, necessarily advantageous, since such a measure may also be very insensitive to treatment effects. Finally, the coefficient of variation may be very misleading when the variable from which it is derived takes negative values.

The relationship between field and laboratory errors

In order to obtain the greatest accuracy in the final data from a given total of experimental resources, it is necessary to know where the major components of the total error occur in the chain of operations field-laboratory-desk. The various components may include: plot variation, sampling, sub-sampling and weighing-errors in the field; sub-sampling and weighing-errors in the laboratory and errors inherent in particular techniques for determining various constituents; copying- and calculating-errors at the desk. The various field and laboratory errors can usually be estimated by duplicating the procedure at every stage. Of course it may not always be possible to estimate each component separately (e.g. sub-sampling may be an essential part of the laboratory determination and this sub-sampling error will be an inseparable part of the error of the determination).

Errors associated with plots and with field sampling and weighing are often the biggest components of total error and it is then futile to improve the general accuracy of the laboratory determinations (as indicated by their reproducibility) beyond a certain point. For example, suppose that field errors are 10% (of the mean) and those contributed by the laboratory are 3%; then, assuming independence of the two processes and applying the usual square laws, the errors associated with final values will be approximately 11%. Clearly effort would be much better employed in reducing field errors.

Our experience of routine laboratory analyses (such as the Kjeldahl method for nitrogen) is that the degree of agreement between duplicate analyses can be kept satisfactorily close for long periods of time. Occasionally, however, gross errors occur (fortunately they are usually easily detected during careful inspection of the results); also, on occasions, very bad 'runs' of analyses occur where all values tend to be much higher (or lower) than they should be or much more variable than usual.

There seems to be no very simple way of dealing with this problem; we do not consider occasional spot checks or the regular insertion of 'standard' samples to be satisfactory. Duplication of all determinations (which would, in fact, usually be quite uneconomic) would only indicate whether reproducibility was satisfactory and would not indicate the presence of bias. Perhaps the best way of keeping the process reasonably under control is to re-submit a complete batch of 20 or 30 samples (per 1000 samples analysed) after, say, a month has elapsed.

Consistent biases have sometimes been found to appear between different analytical laboratories carrying out the same routine analysis on the same samples (there are often strong reasons for believing that this effect is not due to the way in which the original sample is divided between laboratories). The unsuspected presence of such biases may give rise to faulty conclusions when data from many sources are combined.

The number of chemical determinations can often be drastically reduced by bulking all replicates of a treatment before analysis so that a single analytical value is obtained for each treatment. This procedure is

satisfactory if the constituent under analysis is not the variable of prime importance (e.g. unless there is a particular interest in crude-protein percentage and yield, one crude-protein percentage figure for each treatment is usually enough). However, in our conditions, the use of a single bulked sub-sample for the determination of dry-matter percentage is not, in general, satisfactory; one may get considerable drying of the herbage during the sampling of an experiment; and there are also practical difficulties in obtaining a sample of 200–500 g which satisfactorily represents all the replicates.

Although past experience or pilot experimentation may suggest that a marked increase in sampling intensity would be theoretically worthwhile, it must be considered whether the available resources can stand up to the increased requirements. If they cannot, the effect may be, for example, that all samples are mishandled or inaccurately measured because of congestion in ovens or weighing room. In experimental work, a fairly small number of accurate values is likely to be much more valuable than a large number of inaccurate ones (inaccuracies may appear in increased variability and in occasional gross errors).

Recording and computing

Considerable care should be taken in designing the lay-out of field recording sheets. The format must be a compromise between ease of location of the place at which to enter any particular item of information and ease of use or ease of extraction of data for the succeeding calculations. Transference of data from sheet to sheet must be minimized; it is a tedious process and is likely to introduce errors—the incidence of copying errors is higher than is often realized. Very often so called ‘calculating’ errors are due to faulty transference of data between paper and calculating machine.

Advice on how to carry out the usual statistical calculations will be found in many text-books (e.g. Yates, 1937; Pearce, 1953; Cochran & Cox, 1957); much of the computing procedure adopted at the Institute is based on it. A few points are worth emphasizing:

- (a) The normal routine in the Biometrics Department is to duplicate all statistical analyses—the entire calculation is carried out by two operators working independently. This, however, is not always practicable and if only one person is available he should repeat the whole calculation or (preferably) test the results by a different method.
- (b) Careful checking of all transference of data is essential. Reading by one person while another checks is recommended. Alternatively, tabular matter may be added together by rows and by columns on both copy and original and the corresponding totals compared.
- (c) Maximum use should be made of storage and transfer facilities in calculating machines. This minimizes the risk of errors due to writing down results and later re-entering them into the machine. However, in lengthy calculations the results should be noted down at regular intervals; this is an insurance against faulty operating at a late stage of the calculation; it is also a valuable aid to checking.

The magnitude of the variation associated with grassland experiments is such that it is usually sensible to round data to three significant figures before analysis (in this context, the first digit is not always to be regarded as significant, e.g. if all data are in the range 2000–2999, they may in effect be regarded as in the range 0–999 for the purposes of the analysis of variance and hence 2716 would then be regarded as rounded to three significant figures). Such rounding not only reduces the labour of calculation but also reduces the risks of errors due to misreading. In the analysis itself, however, it is wise when dealing with sums of squares, etc., to carry sufficient figures to ensure that the error sum of squares is estimated with four significant digits.

The final presentation of the results should be kept as simple as possible. As a rough guide, all means and their standard errors should, in general, be quoted to three significant figures (see Cochran & Cox, 1957, p. 60 for a more specific rule).

To quote in tables values to too many significant figures not only makes the tables more difficult to comprehend, but may also be misleading.

Lastly, it must be remembered that complex statistical treatment of results cannot make up for poor experimentation and that adequate note-taking in the field is essential to the interpretation of the statistical analysis.

PART II

HERBAGE PLANT INVESTIGATIONS

CHAPTER 5

PLANT PHYSIOLOGY

Specialized techniques used in plant physiology are concerned primarily with living plants; they include methods of plant culture, control of plant environment and measurement of plant growth and composition. In many cases, for example in chemical analysis and the purification of culture solutions for minor-element experiments, the techniques used are well known and are fully described elsewhere (Hewitt, 1952; Paech & Tracey, 1955-6). Modifications have, however, been made in some cases to suit the special conditions of grassland research and some original methods have been evolved.

Plant culture

Pots : soil-, sand- and vermiculite-cultures

Several methods of plant culture are used, depending on the nature of the experiment. For soil culture, clay pots, usually 10 in. in diameter (size 8) and with free drainage have proved satisfactory. Painting them on the inside with bituminous paint renders them watertight and thus reduces the rate of drying. Similarly painted pots containing sand or vermiculite are used if control of nutrient supply is required. Vermiculite has several advantages over sand: it is very light, so that pots can be moved about easily (a 10-in. pot full of wet sand can weigh up to 30 lb); it retains more moisture than sand, and roots can be removed from it with less loss than from the latter. One disadvantage is that it invariably contains soluble inorganic material which cannot be removed by boiling with acid, as it can from sand, without the physical structure, and thus the moisture-holding capacity, being destroyed. Vermiculite is therefore not suitable for experiments in which inorganic nutrients have to be kept at very low levels or omitted. In England, it is also more expensive than sand whilst after about 12 months' use its structure deteriorates so that it cannot be used again. However, in places where a local source of fairly pure quartz sand is not available, vermiculite would probably be cheaper to import than sand, because of its very light weight; in general, the advantages of using it far outweigh the disadvantages.

When smaller pots are required, plastic ones are better than clay as they are lighter and less bulky: being non-porous, they retain moisture better. The necessity for labels can sometimes be obviated by using pots of different colours. Outsize pots, made from large drainpipe sections each

containing about a cubic yard of soil, are also in use as containers for plants growing under sward conditions, on which frequent detailed observations are made (Plate 2). While with these pots the microclimatic conditions, including soil moisture, are undoubtedly not identical with those in the field *sensu stricto*, their range probably being greater, the greater ease of observation enhances accuracy to an extent sufficient to outweigh this disadvantage.

Split-root technique

A special culture method has been devised for growing grass plants with their seminal and adventitious root system separated and details of it have been published (Williams, 1959). In this method a plastic pot with a glass tube heat-sealed into a hole in its bottom is supported within a larger pot, through whose drainage hole the glass tube projects. The seminal root (about 1 cm long) of a seedling is threaded through a small hole in the lid of the inner pot and sealed there with wax of low melting-point, after which the top is fitted on to the pot and the dry vermiculite filling both pots is soaked with water; the lid of the smaller pot has a short tube sealed into it for this purpose. The system illustrated in Fig. 1 has worked well, with no leakage of water or nutrients from one pot to the other. Various sizes of pot have been used, ranging from a 40-ml pot (the smallest available) inside a 335-ml pot (the largest plastic pot then available) to a 335-ml pot inside a 10-in. clay pot.

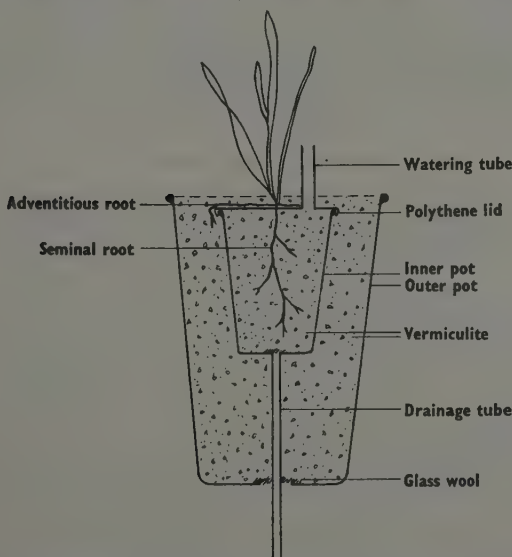


FIG. 1. Sectional diagram illustrating the technique for growing grass plants so that the seminal and adventitious root systems are separate.

Solution culture

Solution cultures are not now much used. Wide-necked bottles of soft glass, or wide-necked flasks of resistance glass, are the best culture vessels for either grasses or clovers. Tops made of paraffin wax, cast in a plaster mould, have been found easier to make and more satisfactory in use than either waxed corks or waxed plaster tops.

Mist culture

Mist- or spray-culture-techniques have been used for experiments on the effect of pH on lucerne, where it was necessary to use solution culture but inadvisable to use standard water-culture techniques, because lucerne does not develop a normal tap-root system under those conditions. The following method is now used for growing plants for use in root-respiration studies. Seeds are sown on small 'sieves' made of short pieces of polythene tubing with terylene net stuck on one end. These are supported by a perforated cork mat floating on dilute culture solution until the seeds have germinated, when they are transferred to the perforated top of a tank bearing a spinning disk atomizer, which provides a mist of culture solution. By this means, roots are produced which can be expected to survive indefinitely when the plants are transferred to a similar, but smaller, chamber for measurements of root respiration.

Culture solutions

Culture solutions used for sand/vermiculite or solution-culture experiments are usually compounded specifically for the experiment in hand. Of the 'classical' solutions, van der Crone's has been found the most useful. Except when this is used, iron is always added as the ferric chelate of ethylene-diamine tetra-acetic acid.

Environmental control

The simplest form of environmental control consists in the provision of a wire cage for the protection of plants from birds; the next stage is the use of glasshouses to give protection from weather and some temperature control. Artificial lights within the glasshouses can give some control of light intensity and, in combination with dark blinds, complete control of daylength. Finally, the use of an environmental room or cabinet can give, theoretically at least, complete control of all environmental factors. All these facilities are available in some degree at the Grassland Research Institute.

Bird-proof cage and glasshouses

The wire cage is simply a tubular steel framework supporting 0.5-in. galvanised wire netting, adjoining the glasshouse site. There are advantages in having the cage directly accessible from the glasshouse, so that plants need never be exposed outside one or the other. The glasshouse is divided into compartments, each 20 × 10 ft, and each entered by a sliding door in the north (10-ft) wall. Heating is by low-pressure steam and each compartment has its own thermostat which is provided with a sufficiently long lead

to allow it to be placed anywhere within the compartment. Electricity, water and drainage are provided in each compartment. There are no fixed benches, plants being supported on movable trolleys; some of these are of tubular steel with tops of reinforced plate glass, others are of slotted angle steel or aluminium, with tops of expanded metal. The most convenient size has been found to be 2×3 ft, with tops 2.5 to 3 ft high.

Supplementary lighting and day-length control

Mercury-vapour fluorescent lamps with internal reflectors (400 watt) are used to supplement the low natural illumination during the autumn and winter. These provide light of high intensity without themselves being sufficiently large to obscure much natural light. They are relatively expensive to install. Lighting equipment for the extension of short natural photoperiods is provided by a combination of fluorescent and incandescent lamps, generally controlled by time switches; automatic control of day-length is achieved by the use of trolleys equipped with a device bearing lights and dark blinds which are drawn up from below plant level so that they throw no shade on the plants while the latter are uncovered. The description which follows was published in the *Journal of Horticultural Science* (Langer & Canaway, 1958): thanks are due to the editors of that journal for permission to reproduce it here.

The device (Figs. 2 and 3) is fitted on an ordinary glasshouse trolley (2.5 $\times$ 4.25 ft) made of angle iron or slotted-metal material, which is continued above into a framework about 2.5 ft high. The top of the framework is faced all round by a double strip of sheet iron approximately 4 in. wide; this forms a light-proof trap into which the top edge of the curtain fits when it is fully drawn up. The curtain itself is made of black material (Bolton twill) and is suspended from a tubular steel frame which surrounds the trolley.

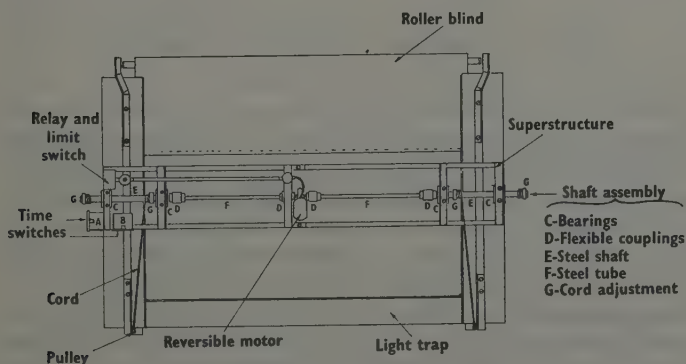


FIG. 2. Daylength control: plan of apparatus.

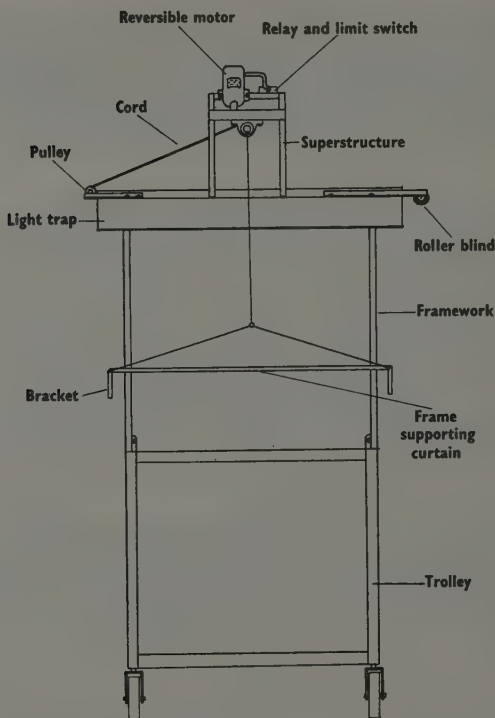


FIG. 3. Daylength control: front elevation of apparatus.

The superstructure mounted above the framework contains a reversible motor and a steel shaft assembly which act as the raising and lowering mechanism. The shaft assembly consists of two steel shafts (E) mounted at either end of the superstructure so that approximately 4 in. of their length project outwards. The shafts rotate in bearings (C) and are connected by flexible couplings (D) and short lengths of steel tubing (F) to the final shaft of a geared, reversible motor which is mounted centrally between and in line with the shafts. The curtain frame is suspended by two cords from the ends of the steel shafts (G) on either side of the superstructure.

When the motor rotates, the cords are wound around the shaft and the curtain is raised. In a similar way a spring-loaded roller blind is drawn across the top of the trolley by means of two cords attached to the shafts between the bearings.

The sequence of operations, from the time that the curtain is up, is as follows. When the time switch (B) reaches the 'on' position, the circuit to the relay is closed and the motor is caused to rotate the shaft assembly. As a result the roller blind is released and wound back through the action of its spring. The curtain around the trolley is lowered and when it reaches a point below the level of the plants, a cord attached to it operates a limit switch which stops the motor. At the end of the light period the relay is released by the time switch and changes over. The roller blind is drawn across and the curtain rises until its frame presses against a rod attached to the limit switch. This completes the operation and the trolley is now completely darkened.

Inside the trolley two 4-ft 40-watt fluorescent tubes and their ballast lamps are mounted. The lighting circuit is controlled by a separate time switch (A), so that the light period may either be composed of full daylight throughout, or preferably in all comparative photoperiodic studies, of a standard period of natural daylight supplemented at either end by artificial light of any desired duration.

The apparatus is kept permanently in a glasshouse. As the light period is usually given during the day, there are only minor differences in temperature between the glasshouse and underneath the dark curtains. The fabric is not rubberized so that air circulation is not seriously impeded. This may have the disadvantage that traces of light might reach the plants, but a light meter sensitive to 0.1 ft c has failed to register any light transmission. However, for plants which react to very low light intensities some improvement might be necessary, even at the expense of reducing the circulation of air. A fan mounted below the plants would help to offset this disadvantage.

Environmental cabinet

An environmental cabinet, providing control of temperature and artificial light, has been built at the Institute (Plate 1); in essentials it follows the main lines of development laid down by the National Institute of Agricultural Engineering, Silsoe.

Plant material is grown in a chamber approximately 5 ft long by 3.5 ft wide by 3 ft high. Artificial light is given by 21 warm-white fluorescent tubes, 5 ft long, with internal reflectors, spaced 0.5 in. apart, suspended above the chamber and separated from it by a double layer of glass with an air gap between. The lights are cooled by fanning air at room temperature along the tubes. The air in the plant chamber is circulated continuously by means of a slow-speed paddle fan placed horizontally below the plants under the floor. The air passes upwards through peg-boarding, which ensures even distribution of the air as it enters the main chamber, and then between the plants which are supported on expanded metal 6 in. above the peg-boarding (Fig. 4). The air is then withdrawn down vents at the top of the end walls, over cooling vanes operated by a refrigerator unit, and is then passed over heaters under the floor, before being redistributed by the fan. To ensure good insulation against fluctuations in the outside temperature, all walls of the cabinet are lagged with 2-in.-thick expanded polystyrene. All the internal walls of the chamber are lined with aluminized

cellulose acetate foil to give greater reflection. Tests indicate that with all the lights running continuously, giving an intensity of 1200–1500 ft c, a temperature of $40\text{--}80^{\circ}\text{F} \pm 1^{\circ}\text{F}$ can be attained. The period of light in each 24-hr cycle is controlled by a time switch, which also alters the setting of the thermostats from day to night temperatures. Temperature changes are sensed by two thermistors which separately control the refrigeration and heating units to give the required day- and night-temperatures. A second cabinet with a larger battery of lights to provide increased incident illumination is now under construction. Except for the inclusion of lights within the plant chamber, and the consequent larger refrigeration system, the lay-out of this second cabinet is very similar to that used in the first one.

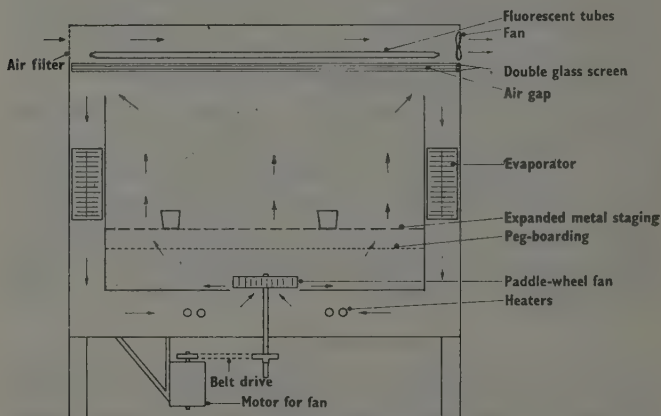


FIG. 4. Cross-section of environmental chamber showing air-flow (arrows represent direction of air-flow).

In conjunction with the supplementary and artificial lighting, incident radiation is measured by photometers [34]* coupled to electrolytic meters [29] (Trickett & Mousley, 1956). An integrated reading of total light intensity incident on the photometer can be obtained with this apparatus. The photometers and meters are recalibrated at intervals against a solari-meter [36], a more stable and sensitive instrument (Trickett *et al.*, 1957).

Measurement of plant growth

Labelling of tillers and leaves

Special labelling methods have been devised in order to follow growth of individual tillers and leaves. Tillers are labelled either by means of small plastic labels attached by coloured nylon threads (in which case the

*Figures in [] refer to Appendix 2.

label bears a number and the colour of the thread can also be used for coding) or by means of plastic rings of the type used on the legs of pigeons (when the colour is used as a means of identification and additional information may be written on the ring with the ink sold for writing on plastic labels). The thread, or the ring, is slipped over the tiller, as low down as possible (Plates 3 & 4). Rings are preferable to threads; they do not become displaced during recording, nor do they become buried in the soil or other substratum. Labels on threads have the further disadvantage that the threads easily become tangled, so that separating them without pulling them off the tillers is difficult and time-consuming. Both systems have been used in pots, and rings have been successfully used in the field. When it is desired to follow the development of individual leaves, or to record leaf production, each leaf is marked with red paint—near the base of the lamina—when it is recorded as dead; the disappearance of older leaves thus gives rise to no doubt. Similarly, if ear emergence is being recorded without individual tillers being labelled, flag leaves are marked with red paint or merchandise tags when the ears they accompany are recorded as emerged. Provided the paint mark does not extend the full width of the leaf, the latter is unlikely to be damaged, though damage is more likely if the abaxial, rather than the adaxial, surface is marked.

Leaf-area measurement

Leaf area provides useful information but its measurement is laborious. The shape of grass leaves results in large errors if areas of leaf prints are measured with a planimeter, as well as making the preparation of prints difficult; consequently methods have been devised for estimating the leaf area from linear measurements made on a sub-sample of leaves. If leaf weights are also measured, the regression of leaf area on leaf weight can be estimated and hence, knowing the total leaf weight of the whole sample, one can estimate the leaf area of the whole sample.

To estimate the area of the leaves in the sub-sample the following procedure is adopted: leaves are measured while fresh, as soon as possible after harvesting; samples awaiting measurement are kept in a refrigerator in order to reduce respiration losses to a minimum, and those on which measurements have been completed are immediately placed in an oven at 100°C and dried (usually overnight) before weighing. The length (L) of each lamina is measured, in millimetres, with an ordinary rule or one made by sticking a piece of millimetre graph paper on to a strip of wood or hardboard; width (B) is measured midway along the leaf by means of a graph-paper rule, or an illuminating magnifier with a built-in scale. The formula for estimating leaf area (A) in mm^2 is then:

$$A=0.905 LB$$

This formula has been found to hold virtually unchanged at various stages of uninterrupted growth of grasses whose leaf shapes are markedly different (Kemp, 1960). If allowance is to be made for assimilation by leaf sheaths, the length and median diameter of the 'stem', which in a vegetative tiller consists almost entirely of leaf sheaths, are measured in

the same way, and the surface area calculated, the structure being treated as a cylinder.

Recently the inclined-plane point quadrat, as described by Warren Wilson (1960), has been used for measurement of leaf area index, height distribution of foliage, and leaf angle on grass swards in the field. The air flow planimeter (Jenkins, 1959) will shortly be used in the laboratory for measurement of leaf area on samples taken from the field.

Weighing of small samples

To facilitate accurate weighing of light but bulky samples, it has been found useful to employ an aluminium container, balanced by a counterpoise, in conjunction with an analytical balance. The container used is a damper pot, supplied by the balance manufacturers.

CHAPTER 6

FIELD EXPERIMENTS

Examination of new herbage plants

A garden is maintained for the purpose of observing spaced plants of introduced species and new varieties. It is arranged in long traverses 24 ft wide separated by permanent grass paths 6 ft wide. As far as possible, legumes and grasses occupy the land alternately. The cultivated area receives an annual dressing of about 50 lb P_2O_5 and 50 lb K_2O per acre. Grass plots receive about 50 lb N per acre repeated two or three times each year.

Seeds are sown, in groups of two or three, in small containers (made from 0.002-in. polythene lay-flat tube 3-in. wide) filled with sterilized soil. The seedlings are singled, and transplanted in May or June. Most of the tufted grass seedlings are set out at intervals of 1 ft, in rows 2 ft apart; large or spreading plants are spaced more widely (e.g. white clover in a square of 3–4 ft).

A single plot of 24 plants is usually sufficient to indicate the main characteristics of a species or variety, including the variation among individuals, although many more plants may be needed for the identification of a variety. It is found useful to have control plots at frequent intervals, whenever appropriate control species or varieties are available.

Spaced plants of perennial species are usually kept under observation for at least 2 years. Observations include: incidence of flowering in the seedling year, date of ear emergence (grasses) or onset of flowering (legumes) in subsequent years, incidence of disease, extent of 'winter burn' and general observations on growth habit, leafiness and vigour. The date of ear emergence for each grass plant is defined as the first date on which three ears are partly visible, and the mean date of ear emergence as the date on which 50% of the plants have reached this stage. The spaced plants are cut once or twice in the year of planting. In the following year they may be cut once in early spring. Each group of varieties is then allowed to grow uninterrupted until the latest variety in the group reaches the mean date of ear emergence (or onset of flowering). The plants in one-half of each plot are then cut, while the remainder are left to flower. This second set of plants is cut after flowering. The two sets of plants are then cut alternately, at varying intervals, during the following 9 or 12 months. This scheme ensures that there are always some 'active' plants under observation.

The herbage cut from spaced plants is sometimes weighed in order to supplement visual observations of vigour. If the weather is dry, fresh weight only may be recorded: in variable weather, a sub-sample is taken from the produce of each plot for dry-matter determination. Yield is expressed as a mean per plant-space: but if some plants are missing for reasons unconnected with genotype the yield of a plot is divided by the

residual number of plant-spaces. Spaced plants of erect or tufted form are cut with a sickle or with hand shears. In most instances the foliage is drawn upright and severed 3 in. above ground level. Rhizomatous or stoloniferous plants are cut with hand shears, usually at a lower level than erect plants.

The weight of herbage produced by widely spaced plants is a measure of the inherent vigour of a variety. As such, it is useful in planning rational sward trials. For instance, if varieties of a grass are to be tested in a mixture with a legume, this may indicate a need to test them at more than one seed rate.

A species or variety which shows some outstanding characteristic graduates to a sward trial. At this early stage, a group of species or varieties is usually subjected to contrasting treatments which might be expected to have some relevance to the habit or rhythm of growth observed in the spaced plants. It is also considered important in most instances to study persistence in the presence of the grazing animal.

Design and management of small-plot experiments

Replication

This varies with the degree of precision required, but in the majority of small-plot experiments the number of replicates established at one time is 2–6. If the experiment is concerned with treatments which interact strongly with annual variations in climate, 2–4 replicates are established in each of two or more years. Comparisons which are not greatly influenced by climatic conditions, and those which extend over many years, are often made in 4–6 replicates established simultaneously.

Size and shape of plots

In the typical small-plot field experiment, plot size and shape are determined largely by the technique for estimating yield, by whether plots are to be grazed individually or in groups, and by the area which must be discarded to avoid border effects. Irrigation equipment may also determine plot size. The effect of plot size and shape on crop variability is a secondary consideration.

Plots sampled only when mown. If measurements of herbage yield are confined to those occasions on which the whole plot is mown, the width of the plot is determined by the width of the mower and by considerations of border or edge effect. The motor scythe [2] fitted with a 2-ft cutter bar and with wheels set to the narrowest track requires only 2 ft in which to operate: so does the 17-in. lawn-mower with rear-roller drive. After allowing for deviations from a central course, the plots may be no wider than 3.5 ft. The motor scythe fitted with a 3-ft cutter bar requires a wider plot. If edge effects are large, plots may need to be as wide as 6 ft in order to accommodate a single swath.

The irrigator (p. 118) spans 12 ft and travels 18 ft, and this area is sometimes divided to give 4 or 5 sub-plots 12 ft long and 4.5 or 3.6 ft wide: these are sampled, respectively, with the 3-ft or 2-ft motor scythe. In other

experiments where a single swath is cut from each plot, the plots may be up to 24 ft long.

Sometimes, as when a strong wind is blowing or when the herbage is lodged, it is obviously necessary to mow all samples in the same direction. Even when the need is less obvious, all plots within each replicate are mown in the same direction.

There is a minimum limit to the area required for access between blocks of plots, whatever the plot size. This requirement is lower when the plots are long and narrow. Four replicates of long plots require less access than 6 replicates of shorter plots of the same width and take less time to sample. These considerations often outweigh the advantage in precision which might be gained by having 6 replicates of slightly smaller plots (if the total area is limited, the proportion of plot to access area is smaller in the latter case).

Plots sampled when grazed. Where plots are to be grazed and samples taken to estimate the quantity of herbage available at the beginning of each grazing period, it becomes necessary to use plots that are large in relation to the sampling area. Since a fresh set of random sites is sampled on every occasion, the total area sampled in the course of an experiment may be quite large, and the 1/1000th to 1/200th acre plots used for mowing treatments prove too small. The minimum convenient size of plot depends on the equipment used for sampling; it also depends on whether the plots are to be grazed separately or in groups. For grouped plots under common grazing treatment it is usually between 1/100th and 1/50th acre. A border 6 ft wide is sown round each group so that the excessive trampling and the accumulation of droppings which occur around the margin of a grazing enclosure do not affect the plots.

The behaviour of grazing animals in small enclosures is likely to be abnormal. Where sheep are used exclusively, the minimum satisfactory size for a paddock is about 1/20th acre. However, when it is desired to introduce the effect of cattle or mixed stock, each paddock needs to be at least 1/6th acre. In screening a large number of herbage treatments against a background of grazing by mixed stock it is therefore almost inevitable that plots should be grouped for common grazing. In such experiments the plot size is consequently more often determined by sampling technique than by the paddock size.

In the study of grazing treatments *per se* it is not always necessary to have large plots. Certain aspects of grazing (e.g. time, frequency and duration, and class of livestock) have been studied in small plots by employing fences to keep stock out rather than in. Stock are kept in an accommodation paddock with controlled access to each part in a compact array of small plots. At any moment they range both the paddock and any plots which are due for grazing. The paddock has only to be large enough to carry one or two cattle and a few sheep whenever there are plots due for grazing. The cattle can be excluded from certain plots by wire strands while sheep still have access to them, so that one plot can be grazed by sheep alone while another is being grazed by sheep and cattle together. If other plots are to be grazed by cattle alone, a second accommodation

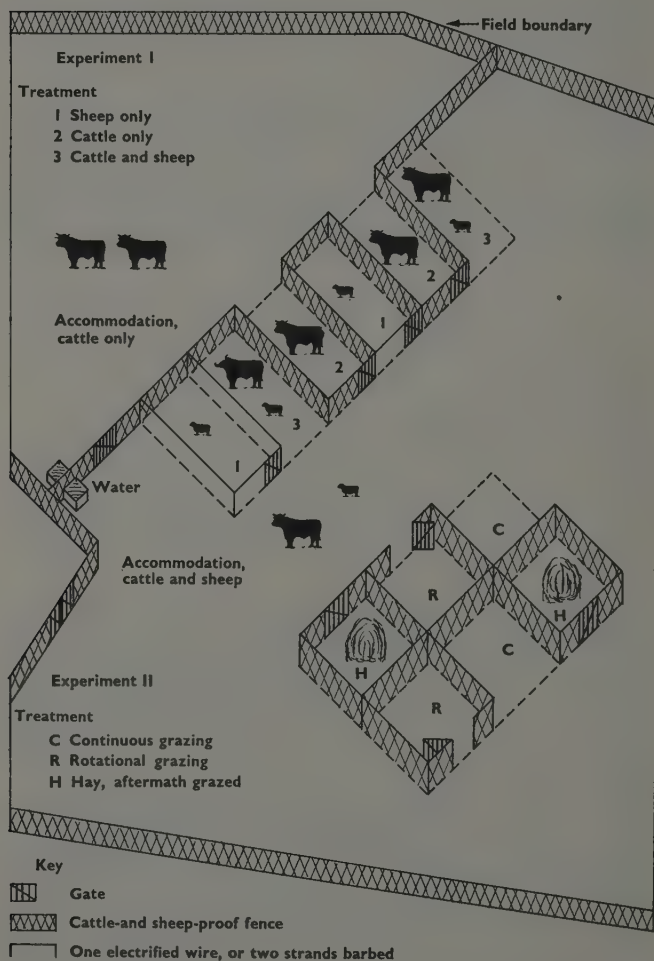


FIG. 5. Examples of use of enclosure technique to compare grazing treatments in small plots.

paddock is made with access at the opposite end of such plots. Fig. 5 shows examples of the method, which can be adapted in many ways. In ecological studies of old pasture, where the area suitable for plots is often limited, this method has proved valuable.

General management in screening experiments : alternate mowing-and-grazing technique

There are two obvious shortcomings in the common grazing of a group of small plots. One is that the livestock frequently show a preference for certain types of herbage. The other is that plant nutrients are not returned to each plot in the droppings in proportion to the herbage removed. Differential grazing of small plots may lead to erroneous and exaggerated estimates of differences in productivity, in so far as regrowth depends on the intensity of defoliation. The random return of plant nutrients, on the other hand, will tend to obscure differences in productivity over a long period. In order to minimize the effect of these two factors (differential grazing and indiscriminate return of nutrients), the general treatment in small-plot trials is usually one of mowing and grazing in alternate periods, with surplus herbage also mown immediately after each spell of grazing. In the first place, intermittent mowing often reduces differences in palatability. In conjunction with mowing of residues, it effectively reduces the cumulative effect of differential palatability. In the second place, the mowing and removal of alternate crops of herbage reduces the rate at which transference of fertility within the experiment might affect the results.

This form of management is not far removed from good practice in countries where a large portion of the annual production of herbage is mown for conservation. While in practice some of the plant nutrients removed in the mown crop might ultimately be returned to the grassland, it is not considered important to reproduce this effect in experiments with ley plots, since these normally have the further general treatment of adequate regular dressings of mineral fertilizers.

The scheme of alternate mowing and grazing is applied in one of two ways. All replicates may be treated alike; or half may be mown and half grazed at any one time. If all replicates are treated alike, then in order to have a more or less continuous estimate of herbage production, the sward is sampled before each grazing, as well as when it is mown. When two sets of plots are grazed and mown alternately, a composite estimate is obtained from the mown herbage only. Sampling when the plots are mown is generally less laborious than pre-grazing sampling, and the estimate of yield is obtained from a much larger sampling area. Moreover, the plots do not have to be any larger than they would be for an all-mowing technique.

In factorial experiments it is sometimes possible to arrange that the factor which has the greatest influence on palatability is studied in main plots, each of which is fenced, and other factors in sub-plots, which are grazed together. In simple trials of varieties or seeds mixtures a whole set of 3 or 4 replicates is sometimes laid out in a single enclosure.

Estimation of yield and composition of forage

Quantitative measurements of herbage in swards are made for the purposes of estimating: (i) relative rates of growth in different swards; or (ii) actual yield of forage accessible either to the mower or to the grazing animal; or (iii) the quantity of herbage grown on, or consumed from, a pasture in a given period.

Relative data (i) often suffice in the screening of seeds mixtures and varieties of herbage plants. More precise estimation of accessible forage (ii) is needed in order to evaluate cultural treatments (e.g. to decide whether response to manures, or yield of out-of-season pasture, justifies the treatment). An estimate of herbage consumed (iii) may be used in conjunction with an estimate of the quantity on offer, to indicate what proportion of the accessible forage is utilized: where several swards are grazed together, such estimates may be used to indicate *relative* palatability. In a given pasture, herbage consumption can also be compared with animal performance, to estimate the feeding value per unit weight of forage consumed.

Cutting machines

Much of the field sampling is done by powered machines. It will be convenient to describe these at this point, since reference will be made to them in the description of cutting techniques which follows. The machines fall into three categories, according to whether they are used (a) to simulate field-scale mowing, (b) to simulate grazing at levels varying from 0.5 in. upwards, or, (c) to cut at ground level.

(a) Machines to simulate field-scale mowing

For the purpose of measuring crops normally removed in practice by mowing, a motor scythe [2] is used. This machine has a reciprocating knife and a 2-ft or 3-ft finger-bar. The cutter-bar assembly is mounted forward and centrally, and the knife is actuated by a central rocker arm. This arrangement makes it possible to cut a swath which has two clear-cut edges, without trampling the crop. The machine is designed to cut at a fixed level, about 2 in. above ground.

(b) Machines to cut at various levels

For cutting and collecting herbage from short swards, and for cutting at different levels, several types of lawn-mower have been tried. The shearing action of a helical blade and cutter-bar is preferred to the scything action of a free-spinning knife, largely because the former action cuts more cleanly and the botanical constituents of the sample are more easily identified. Machines with scything action which collect the cut material do so by suction. While they do not always collect all the herbage they cut, they tend to collect more soil and debris than do machines with a shearing action. The most useful sort of machine in this group is one with a cylinder of fairly large diameter and with no roller in front of the cutter-bar. For light work a lawn-mower with a 17-in. cutter-bar is used. This normally has a roller in front of the cutter-bar, but caster wheels (standard accessories) are fitted in its place for field sampling.

All machines in the lawn-mower group have one serious deficiency compared with hand shears: in herbage longer than about 1 in., the edges of the swath are ill-defined, and the tendency is usually to gather herbage from outside the intended sample area. The bias increases with the length of herbage.

(c) *Machines to cut at ground level*

Two types of machine are in use to cut the herbage from small sampling units at, or near to, ground level. One was designed for hedge trimming, the other for sheep shearing. The standard hedge trimmer [1] has a reciprocating knife with a cutter-bar 12 in. long. The cutting head has a built-in electric motor powered by a portable generating set (or a generator mounted on the motor scythe [2]). The motor is housed at one end of the cutter-bar assembly. An attempt has been made at the National Institute of Agricultural Engineering to convert the standard motor housing to drive a forward-mounted 6-in. cutter-bar. Prototypes of the conversion have been moderately successful, but further development has been postponed, pending results from the use of the sheep shearer described below.

Two modifications have been made to a number of the standard-model hedge trimmers in regular use: both were kindly undertaken by the manufacturer. The standard reciprocating knife has been replaced by one with teeth as long as those of the stationary cutter-bar (normally the latter has a lead of about 0.25 in., but this is sufficient to prevent the cutter from penetrating a grass sward). In some machines the length of the whole cutter-bar assembly has been reduced and a special bottom cover-plate made to blank off the section nearest to the motor. This modification leaves an effective cutting width of only 6 in., which reduces stress on the motor: narrow sampling units also have a relatively low coefficient of variation.

The hedge trimmer requires very frequent overhaul and replacement of cutter-bar assemblies. Given this attention it has proved moderately successful, although expensive to maintain.

The sheep shearer [3] has been introduced more recently and the technique for using this machine is still being developed. It appears to be less difficult to maintain in good cutting order, and, because of the smaller scale of its components, it is capable of cutting closer to ground level than the hedge trimmer. It has been used in conjunction with a frame to cut sampling units 6×1 ft, and also to cut long units, no wider than the shearhead itself. The head used is of the Australian pattern, 3 in. wide, fitted with comb. The cutter is actuated by a flexible drive from a portable engine [3]. The clutch unit for the shearer can be mounted on, and driven by, either the motor scythe or the hedge-trimmer generating set.

Cutting techniques

Techniques for estimating relative rates of growth: the place of the 'pre-trimming' technique

For purely comparative purposes it is seldom necessary to cut all the herbage from a sample area: growth which takes place above a fixed level

is usually positively correlated, to some degree, with total growth. During periods of rapid growth, the portion which can be removed by the motor scythe in group (a) above is representative of total growth. At other times, and also where the swards being compared differ markedly in tiller density, herbage is cut at a lower level by a machine in group (b). In small-plot trials under continuous mowing management, a low-level cut may follow a motor-scythe cut: again for comparative purposes, the presence in the sample of some residues from the previous period can be ignored. As will be seen from the following discussion, comparison of growth over a certain period may also be based on measurement of growth occurring in a part, only, of that period.

Comparative measurements of growth are made with one of two objects in view. The first is to select the most vigorous species, variety or seeds mixture during each part of the year. The second is to compare the vigour of a sward under different methods or intensities of utilization.

The first of these objects is most frequently pursued in small-plot experiments, where the principal measurements are made when whole plots are mown. Plots under alternate mowing-and-grazing management (see p. 37) are trimmed at a uniform level immediately after grazing, so that when they are mown all the herbage that is measured is new growth.

Even when swards are compared in larger plots, on the basis of animal performance, *relative* data of herbage production in the different swards are sometimes desired. The principle employed in getting these data is similar to that used in small plots: the method is widely known as the 'pre-trimming' technique. Small random sample areas in all swards are trimmed to a common level at the beginning of a period and protected from grazing during that period, and at the end of the period herbage cut from the pre-trimmed areas, with the same machine, is weighed. (A fresh set of sampling units is used in each period.) If at least two plots of each sward are grazed in rotation, and a measure taken of the growth which takes place on each plot *between* grazings only, estimates of growth will be obtained for successive or overlapping periods. If the relative growth of the swards during a rest period can be assumed to reflect relative growth during the following grazing period, no more elaborate measurement is needed. The estimate becomes still less direct, of course, but if the rest periods are long in relation to grazing periods, this defect is not likely to be serious. The nearer the grazing treatment of a plot approaches to continuous stocking, the more desirable it is to protect the individual sample areas. Once this is done, measurement can be delayed until the end of a grazing period. Protection during grazing introduces a new error into estimation of actual growth on the rest of the plot (Cowlishaw, 1951), in addition to the effect of pre-trimming, but for comparative purposes this, too, can be overlooked.

The application of the pre-trimming technique to plots under *different* grazing treatments gives relative data still further removed from actual growth. For instance, if the treatments are such that different quantities of herbage are removed by trimming, the recovery on the pre-trimmed areas may be depressed differentially. Within limits, however, regrowth on

these areas will reflect the accumulated effect of the treatments on the vigour of the sward.

Techniques for estimating yield of accessible forage

There is no difficulty in estimating, by sampling, the quantity of herbage that would be removed on a field scale by an agricultural mowing machine. Most of the sampling of this nature is done with the aid of the motor scythe.

The estimation of what is accessible to grazing animals is, by contrast, fraught with difficulties. The level at which samples are cut is based upon a subjective estimate of how close to the ground a particular type of animal can graze with reasonable ease. What is reasonable depends upon the number of hours an animal would need to spend each day in order to get an adequate ration, and upon what constitutes an adequate ration: in certain circumstances a maintenance ration may be all that could be expected, but in others it may be nothing short of a maximum production ration. There is the further complication that accessibility of herbage to the animal depends not only on its position but also on its physical characteristics, factors to which the cutting machine is less sensitive than the animal.

In an attempt to resolve these difficulties the tendency is to cut samples at a lower level during periods of general scarcity than in periods of plenty; and at a lower level for sheep pasture than for cattle pasture. Thus, samples are cut at levels varying with the season from 0.25 to 0.75 in. for sheep-pasture estimation and from 0.75 to 2 in. for cattle pasture. If a machine is used, the type chosen depends in part upon the cutting level, partly upon the length of the herbage (whether it can be raked up after being cut with the motor scythe) and partly upon the sampling area available. In small plots which are to be grazed after sampling, small units are cut with hand shears or with a machine in group (c). A similar procedure has to be adopted whenever it is decided to cut at 0.5 in. or less. If a higher level of cut is admissible, large grazing plots may be sampled with a machine in group (b) (p. 38), or even with the motor scythe (a), since this is a less laborious procedure than cutting with shears or small machines.

In some grazing trials, large samples of the pasture are cut for indoor feeding to estimate the digestibility of the herbage actually being eaten in the field. A flail-type forage harvester has been used to cut large strips from pasture on offer to cattle: the flail action may have an advantage over the reciprocating knife in that it leaves some of the tougher elements uncut.

Techniques for estimating the quantity of herbage grown and/or consumed: the place of the 'difference' method

To estimate growth on a sward, over a period during which no defoliation occurs, all that is necessary is to cut, at ground level, one sampling area at the beginning, and another at the end, of the period. If the sward is mown in the meantime, the quantity harvested is simply added to the difference between the two samples. But if the sward is grazed during the period and the second sampling area protected by cages, it is likely that the growth which takes place on the plot as a whole will be overestimated,

in so far as defoliation and trampling depress growth and the micro-climate of a cage enhances it. The bias can be kept to a minimum by restricting grazing to a short spell at the end of the period. If the experiment is concerned with different grazing treatments, the bias will influence estimates differentially. The greater the expected divergence in growth rate between protected areas and the remainder of the pasture, the greater is the need to move cages within the grazing period so that separate estimates of growth may be made for sub-periods.

An estimate of herbage consumed is derived from the difference between samples of herbage on offer and samples of residual herbage. If the grazing period is of very short duration, these samples are taken immediately before and after grazing, respectively. This leads to a slight underestimation of herbage on offer, since it does not account for growth which takes place during grazing. If a plot is grazed for more than two or three days, the area which is sampled to determine the quantity on offer is protected by cages, and cut when grazing is completed. This introduces into the estimate of herbage consumed the bias already discussed with reference to estimation of *growth* under grazing conditions.

If stock graze below the level at which samples are cut, there will be an additional, negative, bias in the estimate of herbage consumed. In fact, under the conditions prevailing at the Institute an accurate assessment is rarely possible unless the samples are cut almost at ground level, either by hand shears or by one of the machines in group (c). The hedge trimmer is not altogether satisfactory for estimating herbage removed by sheep, but is usually satisfactory where cattle alone are concerned. The labour entailed in cutting herbage samples by hand at ground level rules this method out for all but the most critical assessments, so that the promise shown by the sheep shearer (p. 39) is of considerable interest.

Samples of herbage cut close to the ground, especially those cut by machine, are frequently heavily contaminated by soil. For accurate assessment it has been found necessary to work on the basis of ash-free organic matter.

At best, the difference method can only indicate how much herbage disappears in the course of grazing. This quantity is almost certainly greater than that actually ingested by the experimental animals. It includes herbage eaten by wild animals, and herbage destroyed by trampling. In view of this discrepancy, and of the inevitable bias in techniques for estimating the quantity of herbage actually on offer, herbage measurement must always be an imperfect method for estimating the herbage intake of experimental animals at pasture. Apart from these questions of validity, it is difficult to attain precision in estimates based on herbage samples. The variability of pasture samples is usually high and the sampling fraction necessarily small, and estimation by difference involves the combined variance of two samples.

As a means for estimating growth of herbage, the 'difference' method has fewer faults. It is a more direct method than the 'pre-trimming' method. Where animal production is compared with herbage production in order to assess the degree of exploitation effected by a grazing practice, the 'difference' method is preferred to the 'pre-trimming' method.

Especially is this so where trimming would entail removal of an appreciable quantity of herbage.

Sampling

Plots under mowing or alternate mowing-and-grazing treatment

When the whole of a small plot is mown with a motor scythe or lawn-mower, the sampling area consists of one or two swaths as long, or almost as long, as the plot. Actual examples are: one cut 3 ft wide, 18-24 ft long, or two cuts 3 ft wide, 12 ft long (motor scythe fitted with 3-ft cutter-bar); one cut 2 ft wide, 12 ft long (motor scythe, 2-ft cutter-bar, irrigated plot); two cuts 1.5 or 2 ft wide, 12-18 ft long (lawn-mower). The total sampling area from all replicates varies from 16 to 32 sq. yd.

To sample a large plot or a field before mowing, a motor scythe with a 3-ft cutter-bar may be used to cut 4 or 5 random sampling units about 15 ft long, giving a sampling area of 20-25 sq. yd.

Where losses during conservation are being measured, the entire produce of a large area is weighed during transit to the silo, as the loaded trailers pass over a 15-ton weighbridge. One or two samples are taken from each load for analysis.

Plots under grazing treatment

Compared with the sampling of crops that are mown, the estimation of herbage production under grazing conditions involves: (a) more frequent sampling, (b) more sampling units on each occasion, (c) smaller units and, often, (d) cutting closer to ground level.

Single measurements ('pre-trimming' technique or 'accessible forage'). When large plots are sampled with a lawn-mower for estimation of growth by the pre-trimming technique (p. 40), or by motor scythe or lawn-mower for estimation of accessible pasture (p. 41) the sampling units vary from 9×2 ft to 15×3 ft, and for each estimate 16-20 units, divided equally among replicate plots, are cut. For example, 4 or 5 units are cut in each of 4 replicates, or 8 or 10 in each of 2 replicates. If a lawn-mower is used in the pre-trimming technique, the following procedure is adopted. At the site of each sampling unit, an area about 12 ft long and twice the width of the mower is trimmed to a given level at the beginning of a period, and the herbage discarded. At the end of the period a single cut, 9 ft long, is taken with the same machine, at the same setting, and the produce of this sampling unit is weighed. If the plot is grazed during the period, the area to be sampled is protected by a cage 10×4 ft.

Small plots, i.e. plots of less than $1/20$ th acre, call for smaller sampling units. Examples of sampling units are: 6 ft $\times$ 6 in., 3 ft $\times$ 1 ft and 6 ft $\times$ 1 ft cut with a hedge trimmer; 4 ft $\times$ 1.5 ft and 3 ft $\times$ 3 ft cut with hand shears; 12 ft $\times$ 3 in. and 6 ft $\times$ 1 ft cut with the sheep-shearing machine. Again the number of units contributing to each estimate is usually 16-20, divided equally among replicates.

The hand shears, or smaller machines, are sometimes used, in place of the larger self-propelled machines, to sample larger plots. It is seldom necessary to increase the number of units per sample by more than 25% in

order to maintain precision, so that the sampling area, and the weight of herbage to be handled, are greatly reduced.

Measurement by 'difference' method. Where the quantity of herbage grown or consumed is estimated by the difference method, 'in' and 'out' samples are based on paired sampling units. The sites for each pair of units are fixed before cutting takes place. If for instance 4 pairs of units are to be cut in a plot, 4 sites are taken at random, and 4 more either matched with them visually or sited as close to them as possible. (It is not always possible to have the two units *very* close together, because the presence of a freshly cut area or a cage may attract animals and cause excessive trampling in the vicinity.) One unit of each pair is then allocated at random to the 'in' sample and one to the 'out' sample.

The difference method is chiefly employed where plots are grazed intensively, i.e. for short periods at intervals of at least 3 or 4 weeks. For comparison of swards or treatments, the number of pairs of sampling units cut in the course of a single grazing rotation is usually about 16 per treatment (all replicates included) if the area of each unit is between 1 and 2 sq. yd (lawn mower), or about 24 if the unit is 0.33 sq. yd (e.g. hedge trimmer 6 ft $\times$ 6 in., sheep shearer 12 ft $\times$ 3 in.). More intensive sampling is confined to experiments in which the estimate based on herbage measurement is compared critically with other estimates of herbage intake made within the same plot or group of plots.

Weighing of herbage samples and sub-sampling for analysis

Fresh weight

When a sample is mown with the motor scythe, a sub-sample is taken from the swath before it is raked up. Small grab samples are taken at intervals along the swath, or swaths, care being exercised to take the full width of the swath at each point. From 2 to 3 lb of fresh herbage are collected in this way from each plot and packed in a chip basket or polythene bag. The remainder of the field sample is collected into a canvas sheet together with the sub-sample in its container, and weighed on a pendant spring balance [10] to the nearest 0.1 lb. The balance is tared beforehand with an empty sheet and container. The tare is checked at every 6th weighing, or more frequently in wet weather.

If the sub-samples are collected in chip baskets, these are pressed one into another, stacked with an empty basket on top, and protected from sun and wind by sacks. Baskets are always transported to the laboratory within two hours of sampling. Polythene bags are tied when filled.

If the field sample weighs less than about 5 lb the entire sample is taken indoors and weighed on a platform balance [8] to the nearest 10 g. Short herbage cut with a lawn-mower, or samples comprising small sampling units, can usually be conveniently transferred to the laboratory for weighing and sub-sampling. Loss of weight from evaporation between field and laboratory is then not important, but samples which cannot be weighed soon after cutting are placed in cold storage to eliminate respiration losses. The material brought indoors from each plot is shaken out,

quartered and re-quartered to yield one sub-sample of fixed weight for dry-matter determination.

Estimation of dry-matter content

If the cut herbage is not more than 6 in. long one sub-sample of 200 g is taken to represent each plot. This can be dried satisfactorily in the small tray described in Chapter 20, p. 137. With more advanced or coarse herbage, a sub-sample of 500 g is dried in the large tray. The samples are dried for at least 6 hours at 100°C in a drying oven [26]. They are then weighed on a platform balance graduated in gram units [8].

Preparation of samples for chemical analysis

Sometimes an analysis is made of the dried sub-sample from each plot, but in many small-plot experiments the dried herbage from replicate plots is bulked, before grinding, for chemical analysis. In either case the whole of the dried material is ground, so as to eliminate errors which can easily arise from loss of the more delicate portion if further sub-sampling is attempted at this stage. The ground material is thoroughly mixed and quartered to fill a 6-oz. waxed, screw-cap carton [35].

Preparation of samples for botanical analysis

For botanical analysis in the laboratory a sample is taken from the herbage remaining after the sub-samples have been taken for drying. In most instances the residue from replicate plots is mixed and a sample drawn from the bulk. This sample is analysed by method (b) or (c) below, either immediately or after storage at 5°F (p. 150).

Estimation of relative weights of botanical components

Botanical analysis of herbage is carried out for two distinct purposes. One is to estimate the relative contribution which each component makes to the diet of the animal. The other is to estimate the total weight of herbage present of each species in the field, with a view to assessing its ecological status.

The composition of *herbage harvested* by the mower or by the grazing animal is estimated with a view to evaluating either the component species or, if the relative values of the species are known, the mixture. Material for laboratory analysis is drawn from samples cut primarily for the estimation of forage yield. These may be samples from a crop as mown, or they may be samples taken before and after grazing for estimation, by difference, of the fraction removed. In the latter instance, botanical analyses also indicate for individual species, the proportions of herbage accepted and rejected.

For ecological purposes, an estimate is required, from time to time, of the total weight of active vegetation on each species. (The relationship between this and other characteristics of a species is discussed on p. 49.) While an analysis of samples cut for forage estimation often gives some indication of proportions in the total weight of vegetation above ground, a more precise measure is sometimes required. Samples for laboratory analysis are then cut specially at, or close to, ground level.

Although the portion of the vegetation which is analysed may therefore differ with the object of the analysis, the actual methods of analysis are similar for both. Three methods are used at the Institute, namely: (a) visual estimation of herbage *in situ*, (b) visual estimation of cut herbage in the laboratory, and (c) manual separation of cut herbage.

(a) *Estimation of relative productivity in situ*

This is, of course, a part of normal field observation, and is applicable equally to accessible forage and to total herbage. The more complex the species mixture, the more desirable it is to make estimates in a number of small, random sample areas in each plot or field. The sampling unit most frequently adopted at the Institute is a 6-in. quadrat, since most observers find it convenient for making estimates of relative yield in the fairly dense swards normally encountered in Britain.

In each quadrat an estimate is made of the relative contribution of each species to the 'accessible' or 'total' herbage, whichever is appropriate to the object of the analysis. Ten units are allotted to each quadrat and apportioned to species. In very complex swards these units may be halved. A contribution of less than 0.5 unit in a quadrat is recorded as a trace. In the summation of estimates from all quadrats in a plot, one 'trace' per 10 quadrats is assumed to represent a contribution of 0.1% to the gross yield. Unless a species appears consistently as a trace in more than 50% of quadrats (i.e. contributes at least 0.5% of the bulk of herbage) no adjustment is made to the apparent percentage contribution of major species. The number of quadrats analysed per treatment area varies with the heterogeneity of the sward. For relatively simple mixtures of sown species a total of 40 readings (which may be divided among replicate plots) is usually adequate. For more complex swards this sampling intensity is often doubled, especially if an accurate assessment of minor species is required.

It must be noted that a certain amount of bias may enter into data based upon simple averages of proportions (apart from bias in each subjective estimate). If low-yielding quadrats are dominated by low-yielding species, the estimate of average composition will be weighted in favour of these species. An increase in quadrat size will sometimes remove much of this bias, but it will also tend to increase the error of the observer's estimate in individual quadrats.

(b) *Visual estimation of the composition of cut herbage*

After thorough mixing and careful sub-sampling of cut herbage, a small quantity will adequately represent the field sample. There is little need for replication of laboratory analysis. At the same time, the quantity of herbage that can be hand-separated is also small, even when one has facilities for cold storage. With practice, an estimate of composition which is suitable for comparing any two swards on the same occasion can be made visually on fresh samples spread out thinly on a bench, provided the components are few and easily distinguished.

A sub-sample, 50–200 g, is split into 10 roughly equal parts which are spread thinly in separate areas. Two or three observers make independent

estimates of the composition of each part, apportioning 10 units among the components. Each observer then has only to total his scores for the whole sub-sample in order to arrive at a percentage contribution for each component. Observers do not compare estimates between samples, and a simple average of their estimates is used. However, before a batch of samples is subjected to estimation, a few dummy samples are estimated and then separated, and the components weighed, so that each observer has an opportunity to justify his estimation.

(c) Manual separation and weighing of components

This method has the obvious merit of objectiveness, and it is free from the mathematical bias mentioned under method (a). The quantity of herbage analysed from each sward on any one occasion is determined largely by particle size and ease of identification. Statistical precision is often affected by the time available and the usual practice is to take as much herbage as can be separated into its constituent species by two persons in 20–40 minutes. The longer time is allowed where identification or physical separation is difficult. On this basis the total dry weight of the sample ranges from 40 to 20 g. The separates are dried in small tins and then weighed, dry, on a balance graduated in units of 0.1 g.

Some attempt has been made to use the point frame for estimating relative contributions to the volume of herbage by recording every hit made by the point of each pin. Vertical pins give exaggerated values for species whose foliage tends towards a horizontal position. The use of inclined pins, which removes some of this bias, has been tried, but it is difficult to trace all contacts without unduly disturbing the vegetation before the pin reaches ground level. On the whole, manual separation of cut material appears to be a more efficient technique for estimating the contribution of each species to yield.

Grass seed crops

Selection and breeding for superior forage production has given rise to varieties of herbage plants which tend to yield relatively small crops of seed. Agronomic treatments which affect seed yield are studied at the Institute in small-plot experiments. Among the factors studied are seed rate, spacing and gapping, manuring, and defoliation by cutting or grazing.

In such experiments the individual plots are usually 1/400–1/200th acre. In grazing experiments, larger plots are required and this restricts the lay-out. For sheep-grazing, a split-plot design is used in which the main plots (for grazing treatments) are at least 1/50th acre. Cattle-grazing requires still larger paddocks, and places a further restriction on lay-out. The cattle graze long strips, which embrace several replicates of other treatments, and in which they are contained by temporary electrified fences.

Methods used for following tiller development and for estimating yield of seed are described below.

Estimation of seed yield

With plots of 1/400th acre, a marginal strip is discarded and the remainder of the plot (approximately 8 sq. yd) is harvested. With larger plots a sampling method is used. Random 1-yd lengths of drill, or 1-yd quadrats in broadcast crops, are taken. The total area harvested from each plot in this way is 6–8 sq. yd.

The crop is cut with hand shears and the sample from each plot gathered into one or more sheaves. Each sheaf is then placed head-down in a muslin bag, 36 in. long and 18 in. wide, with tape ties. The sheaf is secured with the butt protruding from the bag, and hung head-down in a loft where air can circulate freely. Seed is threshed in a bench machine with 10-in. rasp-beater drum, and cleaned by hand-sieving and by aspiration.

Observation of tiller development

In the past, estimates of fertile tiller numbers have been made by counting heads in samples from approximately 1 sq. yd per plot. More emphasis is now placed on the performance of individual tillers, and permanent quadrats are established from which information on vegetative and fertile tiller numbers is derived. For continuous drills or broadcast plots the quadrat is respectively a 6-in. length of drill or a 6-in. square. Tillers in the quadrats may be counted, or alternatively all tillers in the selected area can be labelled with 3/16th-in. plastic pigeon rings. This labelling is done as soon after harvest as possible and is continued at monthly intervals. By using a monthly colour code the pattern of tiller formation and behaviour can be constructed. The quadrats are harvested separately from the remainder of the plot. Tillers from ringed quadrats can be separated into age categories. The small groups of tillers are threshed by hand.

Botanical analysis in ecological studies

The techniques described in this section are used to amplify general observations of an ecological nature. There is no question of data obtained by these methods displacing such observations. Frequent observation, with verbal description or scoring, remains one of the most important methods for recording botanical processes in the field. But in so far as the measurement of one botanical attribute helps to interpret other observations, some technique for measuring or estimating that attribute is indispensable.

Weight of herbage

The total weight of herbage produced by a species at the same stage in successive years is only one of the characteristics by which its ecological progress may be assessed. Herbage production may be expressed in terms of absolute weight per unit area, or as a relative contribution to the composite yield, or in both ways. The absolute weight of herbage contributed by a species is calculated from two estimates, one of composite yield and one of the relative contribution made by the species. Relative contribution is estimated by one of the three methods described on pp. 45–7. As is

mentioned, the material analysed may be that collected for estimation of forage yield, or it may be herbage specially cut at ground level.

In some old pastures studied at the Institute, variation in composition is more intense than variation in composite yield. Sampling for botanical analysis has therefore to be more intensive than sampling for determination of yield. A large number of small quadrats is used for estimation *in situ*, or a large number of very small areas is cut to provide material for laboratory analysis. In the latter case the area of each sampling unit may be only that of the opening of a pair of shears or scissors. Estimates of composite yield based on such small sampling units are often subject to considerable bias. On the other hand, it is only when the total sampling area is very small that it is feasible to cut all herbage close to the ground. Although the assessment of a species may therefore have to be made solely or mainly on its relative contribution to a mixture, it will be more critical in the sense that it does take into account the whole of the aerial vegetation of that species.

For ecological purposes the present techniques of sampling may be improved by the cutting of long, narrow sampling units with the modified sheep-shearing machine [3]. Not only will its use reduce variation between samples in yield and in composition, but also a relatively large area can be cut close to ground level.

Plant population and distribution

The dynamics of a plant association cannot be adequately described without some indication of changes in the number, disposition and growth habit of individual plants or shoots of each species, as well as their size. If the number of members is known, their average size can be calculated from yield per unit area, estimated by one of the methods described in the previous section. While the size distribution among these members, and the spatial disposition of each size-group, will affect the competitive ability of a species, a detailed analysis of these factors is rarely feasible. A combination of herbage production data with estimates of (a) plant or tiller density, or (b) ground cover, or (c) specific frequency in random quadrats, has usually to serve as a basis for describing botanical changes. Techniques for estimating these attributes are described below.

Plant or tiller density

An estimate of plant or shoot numbers has the virtue that population density is an objective measurement. It is, however, one which can be made on a very small area in a given time. The nature of soil variation normally dictates that a relatively large area be studied, so that sampling is the rule. With a small sampling fraction the use of sampling units of minimal area is imperative.

For counting seedlings in young leys, or plants in tufted vegetation, these conditions are less stringent than they are for counting shoots in dense, heterogeneous vegetation. While 6-in. or 12-in. quadrats are normally used for the former purposes, still smaller quadrats are used for counting shoots, provided that the margin of a quadrat can be defined satisfactorily: a 3-in. quadrat usually meets this requirement, but a 6-in.

quadrat is sometimes more convenient in taller herbage. In all these cases estimates are based on 10–20 quadrats per plot on each occasion. In studies of seedling development or mortality, permanent quadrats or whole microplots of up to 1 yard square have been mapped at intervals with the aid of a pantograph.

Ground cover

Ground cover is defined as the area occupied by a vertical projection of each species as it grows. It is expressed, for each species *independently*, as a fraction of the whole area. No account is taken of the overlapping foliage of the same species. For this reason, and because some plants have more erect stems and foliage than others, ground cover is only loosely related to weight of herbage.

Ground cover is useful for indicating the progress of a species so long as the growth habit of that species is not grossly modified by the agronomic treatment of the sward as a whole. Such modifications can occur, without necessarily altering the species' ecological status. For instance, some plants which form prostrate rosettes in low swards become sub-erect in tall swards, thus decreasing in ground cover; yet they may remain equally successful as plants. (From an agronomic standpoint, such plants may become more valuable as their ground cover decreases if, at the same time, their herbage becomes more accessible to livestock.)

In the study of botanical divergences brought about by contrasting agronomic treatments, this plasticity of plants complicates the interpretation of 'ground cover'. A partial solution to the problem is to revert, periodically, to a common agronomic treatment of all plots, or of sample areas within plots. This tends to restore the relationship between the ground cover and ecological status of a species. In experiments on botanical associations of various kinds at this Institute, mowing 2 or 3 times at short intervals to a level of about 2 in. brings both tall and short swards into a condition in which ground-cover analysis describes reasonably well the relative status achieved by most of the species under the experimental treatments.

Visual estimation of ground cover, although subjective, is also a rapid method. It is used extensively at the Institute. Emphasis is placed on the desirability of having successive estimates made by the same observer, and of training observers so that their standards differ as little as possible. The choice of quadrat size is probably important in achieving the latter object. In all but the most heterogeneous swards, most observers find a 6-in. quadrat a satisfactory unit on which to focus for visual estimation of ground cover.

In a few experiments permanent quadrats have been used for estimating changes in the ground cover of sward components, but more frequently a fresh set of random sites is taken for each successive analysis. From 10 to 20 quadrats are examined in each plot, according to the complexity of the species-mosaic and the number of replicate plots. Few investigations warrant the examination of more than 40 or 50 6-in. quadrats, spread over all replicate plots, for each treatment on each occasion.

Each species is considered in isolation, and the fraction of the quadrat area covered by it recorded in tenths. When a species covers less than 0.1 of the area it is recorded as a 'trace'. A separate estimate is made of bare ground, in similar terms. In the summation of all quadrat readings for one species, a 'trace' is assumed to be 0.05 of a quadrat, unless the species consistently occupies only a trace, when a note is made of its presence only. It is considered desirable to restrict the use of ground cover estimates to comparisons between species of similar growth habit, or to the tracing of changes in single species (or bare ground); and to use the recorded value for a particular species, rather than to summate the fractions for all species and then express each as a proportion of a meaningless 'total cover'—which may vary from less than unity to several times unity. The estimate of bare ground is a useful independent record.

Specific frequency

Quadrats. The frequency with which a species occurs in random quadrats is still less closely related to its productivity than is its ground cover. Yet, as an adjunct to estimates of relative productivity a frequency measurement often has special value, if only to indicate the distribution of a species. So long as a species is present in a sward it has a potential (a) to produce herbage, desirable or undesirable, and (b) to influence production in associated species. Some species exert an influence on the productivity of associated species which is out of all proportion to their own contribution to herbage yield. The density and distribution of such species is quite as important as their yield or ground cover.

Another merit of frequency data is that, given quadrats of appropriate size, they give a good indication of plant density (i.e. number of entire plants per unit area) for many species. For these species, frequency measurements will be more consistent from one time of year to another, and between favourable and unfavourable seasons, than measurements either of production or of ground cover. Any major changes in frequency that do occur will probably indicate changes in the plant population. The recording of the presence or absence of a species in a quadrat is an objective process requiring only the ability to identify species and a little practice.

The size of quadrat used for recording frequency varies from one experiment to another, but may also be varied, with advantage, for groups of species within an experiment. Thus for species distributed sparsely a quadrat of 1 ft or even 1 yd is used, while for some major species, a quadrat of 6 in. or even 3 in. is more appropriate. A quadrat of less than 3 in. has not been used, partly because very few of the mature plants studied would, as individuals, occupy less than this area. Presence or absence of each species is recorded in 100–200 quadrats *per treatment*.

Point quadrats. The point frame illustrated in Fig. 6 is used most extensively to describe the constitution of botanically complex swards. It is used in such a way that the incidence of a species within the narrow circle described by the circumference of each pin is recorded. As each pin passes vertically through the vegetation every species with which it comes into contact is recorded. (The number of contacts between a species and

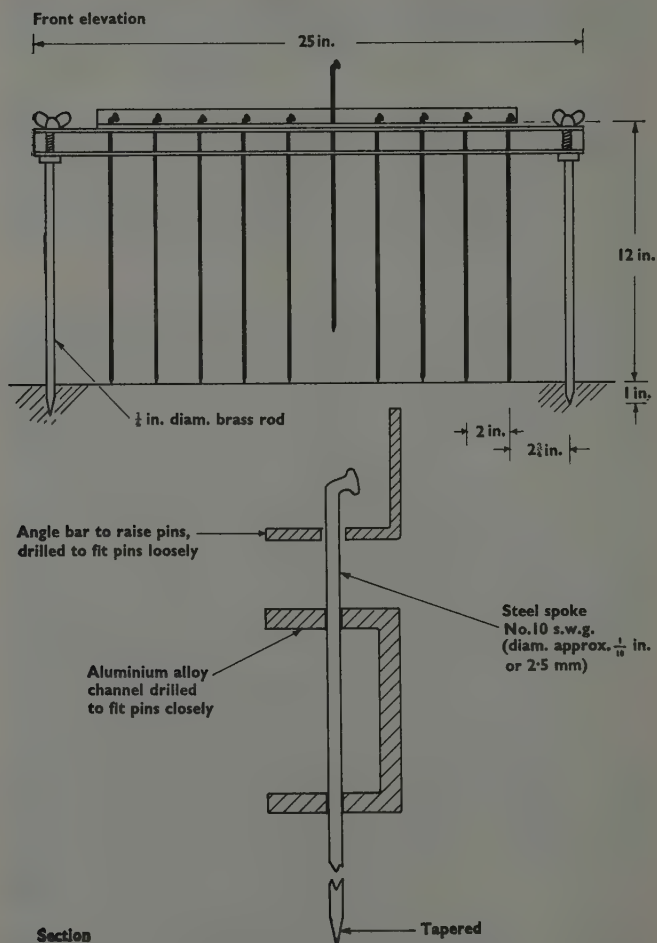


FIG. 6. Frame used for point quadrat botanical analysis.

that pin is ignored.) The pin is not allowed to displace vegetation downwards, each leaf or shoot which is not deflected by it being guided to one side (but allowed to remain in contact with the pin) with the minimum of disturbance to the rest of the sward, until the pin reaches the ground or the base of a shoot.

The data recorded are in fact specific frequency data, but, since the 'quadrat' (i.e. the transverse section of the pin) is so minute in relation to most leaves, the sum of the readings approximates to a measurement of ground cover. The readings are not entirely free from subjective influences, but are more objective than visual estimation of ground cover. Although they have that much in common with other frequency data, 'point-frequency' data have a greater affinity with estimates of ground cover in their application: they give little or no indication of population density or distribution, and their intrinsic value for describing changes from year to year in the status of a species depends on their being recorded when the plant is in the same phase of growth each year.

CHAPTER 7

CLASSIFICATION AND SURVEYING OF GRASSLAND

The plants which are found in grassland are a result of many environmental factors of soil, climate, topography, cultural practices, and their exploitation by man through domestic animals; the natural fauna also frequently affects plant distribution. The appearance of a plant or plant community is therefore a reflection of a particular environment; changes in environment produce changes in the plant community.

It is desirable that the botanical classification of grassland, particularly that of enclosed grassland which has been most influenced by cultural practices, should be capable of reflecting the incidence and intensity of such practices. It is also desirable that the classification should bear some relationship to the productive capacity. Early work on the botanical composition of some of the best fattening and dairying pastures in England and Wales showed that perennial ryegrass was an important species in the majority of cases. Armstrong (1907) using improved methods for assessing the botanical composition showed that, in the midland counties of England, cattle-fattening pastures were preponderantly composed of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) while in the same localities pastures devoted to the rearing and growing of cattle contained considerably less of these two species and more *Agrostis* species. The relative abundance of fine-leaved fescues, *Agrostis* and perennial ryegrass was used by Stapledon (1925) to classify the permanent grassland of Britain. Fenton (1934) observing sward botanical changes in a large number of fertilizer trials noted that, during improvement by these means and under grazing conditions, all plots tended to pass through similar phases of botanical composition; the order of progression with increasing productivity was from fescue dominance through *Agrostis* dominance followed by an increasing contribution of perennial ryegrass in association with white clover. Very similar changes in botanical composition were observed in fertilizer experiments reported by Milton (1947) starting with a *Molinia* or *Festuca-Nardus* grassland association. Recently, Neenan *et al.* (1959) have shown that a strong positive correlation exists between the productivity of grassland and the abundance of perennial ryegrass, white clover and rough-stalked meadow grass.

This ordered sequence of floristic change has formed the basis of classification of permanent grassland and has been used for large-scale surveying of permanent grassland in England and Wales (Davies, 1936, 1941; Stapledon *et al.*, 1952) and its cartographic presentation (Stapledon *et al.*, 1945).

The grassland survey

The surveying and classification of grassland provide the following information:

1. The types of grassland within a specific area or country,
2. The distribution of the individual grassland types,
3. If surveys are repeated at intervals changes in grassland type may be related to changes in management.

There are two major categories of grassland: unenclosed or rough grazing, and enclosed grassland.

Unenclosed or rough grazing

In Great Britain, the rough grazing includes the open hill and mountain pasture, and some chalk downland areas and sandy heaths in eastern and southern England. The botanical composition of these grassland areas is very closely allied to soil and climatic factors. The demarcation of the individual grassland types is fairly distinct and may be accomplished by simple reconnaissance surveys. The following types are readily recognizable on the mountains and moorland of the west and north of Britain—*Molinia* moor, *Nardus* moor, cotton-grass moor, bilberry and heather moors, bracken, and upland bog. In the south and east the unenclosed downland is typically dominated by fine-leaved fescues (*Festuca rubra*, *F. ovina*), although *Bromus erectus* and *Avena* spp. may be locally dominant.

Enclosed grassland

Permanent grassland

In the survey of England and Wales the following types of permanent grassland were identified:

First-grade ryegrass pasture. Perennial ryegrass contributes at least 30% of the pasture by ground cover. White clover is often abundant and other good grasses present include cocksfoot (*Dactylis glomerata*), meadow fescue (*Festuca pratensis*) and timothy (*Phleum pratense*). Crested dogstail (*Cynosurus cristatus*) may be present. Compared with other types of ryegrass swards *Agrostis* spp., red fescue (*Festuca rubra*) and Yorkshire fog (*Holcus lanatus*) are at a minimum. The range of species is relatively restricted.

Second-grade ryegrass pasture. Ryegrass contributes 15–30% of the ground cover. Clover is normally plentiful whilst *Agrostis* and other less productive grasses are more abundant than in the above. The range of grasses is similar to the first-grade ryegrass swards but meadow barley grass (*Hordeum secalinum*) and tussock grass (*Deschampsia caespitosa*) may occur, together with a wider range of miscellaneous herbs.

Agrostis/ryegrass pasture. Ryegrass is present but contributes less than 15% of the ground cover. Clover is present and *Agrostis* is typically the dominant grass. The better grasses contribute less than in the foregoing types whilst red fescue, Yorkshire fog, meadow barley grass, tussock grass, rough-stalked meadow grass (*Poa trivialis*), meadow foxtail (*Alopecurus pratensis*) and sweet vernal (*Anthoxanthum odoratum*) may all be present.

Agrostis pasture. Ryegrass is normally absent. *Agrostis tenuis* is typically the dominant grass whilst the characteristic associated grasses over a wide

range of soil types are Yorkshire fog, red fescue and rough-stalked meadow grass. The presence and amount of meadow foxtail, sweet vernal, crested dogstail, meadow fescue, timothy, tussock grass and meadow barley grass vary with the soil conditions and management. On acid soils the herbage is relatively restricted whilst on basic soils the flora is varied and includes many miscellaneous herbs.

Agrostis with rushes. This is an *Agrostis* pasture with impeded drainage and hence is associated with rushes and sedges. *Molinia* and tussock grass are frequently present.

Agrostis/fescue. Fescue (either red or sheep's fescue) contributes more than 10% of the ground cover but is not dominant. These pastures are frequently associated with acid soils and the clover content is normally low.

Fescue/Agrostis. Fescue (either red or sheep's fescue) is dominant and the sward includes appreciable amounts of both *Agrostis canina* and *A. tenuis*.

Temporary grassland

This is recorded according to the following characteristics:

- (a) Seeds mixture,
- (b) Age
 - (i) first harvest year
 - (ii) second-third harvest year
 - (iii) fourth harvest year and older,
- (c) Percentage of unsown species
 - (i) 0-25%
 - (ii) 25-50%
 - (iii) more than 50%,
- (d) Percentage of bare ground
 - (i) 0-25%
 - (ii) 25-50%
 - (iii) more than 50%.

Pasture type grouping and cartographic representation

The type of pasture existing in enclosed lowland varies from field to field and the grassland area of a district is therefore a mosaic of a number of pasture types. In a survey of any considerable area it is impracticable to examine each field individually, also in preparing a map of manageable scale it is clearly impossible to show field-to-field distribution of sward types. At the same time to allocate an entire area to one pasture type would be to generalize too much. Various pasture 'types' were therefore arranged together to form a pasture 'group', the 'group' being determined by the order of prevalence of the two or three most abundant pasture types.

The grassland map of England and Wales (Stapledon *et al.*, 1945) was prepared after carrying out a reconnaissance survey which was followed by a field-to-field survey of small sample areas. The reconnaissance survey was carried out by workers operating in pairs, travelling by car and inspecting

numerous fields. The main object of this reconnaissance survey was to identify and delineate on maps the different pasture 'groups' by reference to the two or three most abundant pasture 'types' within any area. The period from early autumn to late spring is particularly suitable for reconnaissance surveys in Britain as, in addition to actual inspection and sward examination of individual fields, colour variations, arising chiefly from the differences between species in winter greenness and length of growing season, can be made use of to distinguish pasture types. Using colour variations and working from vantage points a census of fields belonging to different types can frequently be made over reasonably sized areas at suitable seasons. In the reconnaissance survey the scale of the map used was 1 in. to 1 mile (1:63,360). Field boundaries are not marked on these maps but sufficient details of roads, woodland and other landmarks are marked to enable location to be determined with the necessary degree of accuracy.

After having determined the regional distribution of pasture 'groups' on the 1 in. to 1 mile field map, sample areas were then selected within all pasture groups. Each sample area contained between 100 and 200 fields and was examined field by field using maps of the scale 6 in. to the mile (1:10,560) on which field boundaries are marked. In this more detailed survey the individual field was taken as the unit of investigation, and the analysis was obtained by traversing each field on foot. No count was taken of sward variation round gateways, water points and tracks. Such a procedure is satisfactory in most cases, particularly with permanent grassland where the controlling factors have operated for a considerable time, and where field boundaries, originally, often separated areas of different soil types, drainage, aspect, etc.

The field-to-field survey of sample areas was first made in 1939 and was repeated on the same areas in 1946-7 and in 1958-9. Continuous records are available for some 30,000 individual fields.

Each field or sub-division of a field is numbered and the area assessed by a planimeter. These data, together with the results of each survey, are recorded on a Hollerith punched card for analysis.

PART III

ANIMAL INVESTIGATIONS

CHAPTER 8

EXPERIMENTAL PLANNING AND MANAGEMENT

Design and lay-out

The design and lay-out of the experiment is largely determined by the type of trial. A number of factors, such as topography, soil fertility, paddock size, grazing potential of the sward, grazing method, previous treatment of the area, and lay-out in relation to return of dung and urine must be considered. They have been discussed in detail by Brown (1954), by Sears (1951), and by a joint committee of various American societies (1952) and will be mentioned here only when it is necessary to do so.

The duration of an experiment is often determined by its objectives. There is, however, a fundamental problem in grassland research that affects this question. In a field experiment, every treatment effect on the pasture and on the animal may have long-term consequences not measured in a period of time that is otherwise adequate. This may mean that the results of experiments covering parts of the year cannot be combined to give a true picture for the whole year. The form in which information is required will determine whether it must come from a long-term or a short-term study. The number of animals to be used is to a large extent a statistical problem: there are, however, other considerations. In behaviour studies, for example, the flock size necessary for a particular behaviour pattern may exceed that required for statistical purposes.

Replication, or at least random allocation of plots to treatments, is desirable in order to overcome differences in soil type. Animal-grazing investigations require a large acreage for each treatment, so as to allow seasonal production to be measured with adequate stock numbers. The actual size of the experiment needs to be limited, or it becomes unmanageable. With sheep experiments, size presents no particular difficulty; but with cattle a much larger area per head is required; and randomized lay-out has not in all cases been successful. Hughes (1951, 1952) used two such designs in the evaluation of seeds mixtures and noted that experiments of this nature should at once take into account statistical validity and practical management of the livestock. Before the advent of modern forage-harvesting equipment it was impracticable to conserve surplus growth as hay or silage.

In view of management difficulties, Tayler & Large (1955) used two adjacent 6-acre paddocks, each 100 yd wide on a ryegrass sward, and two

adjacent paddocks of equal area and dimensions sown to timothy/meadow fescue. Grazing was controlled by electric fencing within the paddocks, each of which was treated as a unit with a single group of stock remaining on it. The differences in yield were sufficiently large to give confidence in the result, in spite of the lack of a replicated and randomized lay-out; but it was noted that it was possible, as well as desirable, to increase randomization without sacrificing flexibility of management.

Self-contained farmlet trials, as used by Alder (1955) and Alder & Redford (1958), are valuable for following up results from more detailed experiments, for preliminary trials and for demonstration; but they do not give statistically valid findings.

At the other extreme, a single-cow plot technique has been used by Cox *et al.* (1956), while tethering and movable folds were used for sheep by Jones (1937). Cox *et al.* state that the advantages of the single-cow technique are that it allows a conventional replicate experiment to be carried out on the smallest possible area. As well as making the experiment more practicable, it minimizes the component of between-plot variability arising from fertility trends. The between-cow variability is also reduced by avoiding competition between cows when grazing. Such factors as herbage intake can also be measured and related to production.

Many of the difficulties of management are due to the fact that the farmer has a more or less constant number of domestic stock on his farm, whereas growth of grass is seasonal and dependent upon weather. Research into specific problems frequently overlooks the management of the farm as a whole and the planning of grassland management. For this reason in two experiments at the Institute (H.138 and H.176) an attempt is being made to fit self-contained treatments into replicated and randomized designs. In both experiments four treatments are laid out in randomized blocks with 3-fold replication. In H.138, 4 or 5 sheep are rotationally grazed around a treatment group of four plots, each $\frac{3}{16}$ th acre; while in H.176 two cattle are maintained on each group of four 40×60 yd plots, and the plots are randomized throughout the block. In both experiments the management is so designed that hay and silage can be conserved from the plots and fed back to the appropriate animals. In H.138 the sheep are maintained throughout the year, weaned Scotch Half-bred ewe lambs being brought into the experiment each September and remaining there for one year when they join the farm breeding flock and are replaced by a new group of weaned lambs. In H.176 all calves are reared uniformly. The animals enter the experiment when about four months old and remain on their respective treatments until slaughter. Although plots in these experiments are rectangular, a regular shape is not essential if they are accurately surveyed, and experience in the handling of small plots with modern forage-harvesting machinery has largely overcome the difficulty of herbage conservation. It is necessary to leave races or paths 15–25 ft wide for access to plots. Gateways must allow entry of farm machinery for grass cutting and harvesting and for the application of fertilizer; 10- or 12-ft gateways have been satisfactory. The shape and size of plot must also be considered in relation to the efficient working of machines, this aspect being more important than the minimum paddock-size upon which

stock can be grazed. The plots of H.138 (21×43 yd) are the smallest of this type that we have yet used successfully.

Various other designs can be considered for specific requirements (in experiments using identical twins for example) but whatever the design it is evident that there are two contrasting requirements. First, farming practice must be borne in mind, and, second, statistical accuracy is essential. The lay-out and design of an animal-grazing trial must obviously be a compromise, whose details are determined by the nature of the problem.

Grazing management

Grazing management is necessarily subjective. It is difficult, particularly in the climate of the British Isles, to predict future growth. Stocking has to be matched with the pasture available in order to measure the production of individual animals and the output per unit of land. Before an experiment is started, one must decide whether stock numbers are to be varied or whether excess herbage is to be harvested. In experiments involving annual production, as opposed to the seasonal grazing trial, it is usually necessary to cut some of the experimental area for silage or hay during flush periods of growth, unless purchased feed is to be used. Thus, although stock numbers can, or need not, be varied in annual self-contained experiments, a certain area must be set aside for conservation. In seasonal grazing trials the stocking rate can if necessary be varied and the herbage grazed as it grows. Stocking rate appears to be the most important factor of those which the agronomist can control. The ideal appears to involve at least two rates with some predetermined method of adjustment, either by altering stocking rate or by cutting. Alternatively, one might work with a fixed number of animals per treatment, and vary the plot size. In all cases, however, where small differences are being sought, different stocking rates on different treatments will give results which are difficult to interpret.

Fencing and watering

Fencing must control stock efficiently if significant results are to be achieved. For beef cattle, 3-strand barbed-wire fences, with heavy creosoted posts every 3 or 4 yd having struts at the corners and at intervals of approximately 50 yd for straining, have proved satisfactory. If sheep and cattle are grazed together 3-ft wire-netting with one or two strands of barbed-wire above it and a plain wire at ground level to which the netting is secured has been found necessary. This type of fence is desirable if cattle are to be prevented from grazing under or over the fence line. For sheep, wire-netting (with a bottom wire to which the netting is secured) and light wooden stakes are satisfactory.

Electric fencing is satisfactory for restricting bullocks to portions of larger plots but it is not effective for permanent plot divisions, as it is with dairy cows (MacLusky, 1955; Cox *et al.*, 1956). The rationing of herbage to beef cattle must not be too strict and the fence must be kept in efficient working order. A bent post with the wire extended towards the animal has

been tried in an effort to stop rubbing (Hughes, 1953), but it is not effective in controlling the rogue animal, neither is a 2-strand electric fence. The most effective control has been obtained with electrified barbed wire, a method which might be considered for permanent control on larger plot areas. Electric fences have been used for sheep but a movable fence of wire-netting and metal stakes (see Plate 5) gives more effective control, is portable and easily erected.

The filling of water-tanks can be laborious, but it is necessary where a piped supply is not available. With a piped supply, the use of polythene tubing and portable water bowls or troughs is effective. A small trough or tank fitted with a ball valve, or water bowls [18]* fitted to channel-iron frames are used. The former has been used for sheep and cattle, but the latter are used only in cattle experiments. The drawbacks to the use of alkathene tubing are that water can get very hot during the summer (this might be a disadvantage in hot climates), and the water in it freezes easily, but frost does not crack the piping. Both these faults can be remedied in this country by burying the tube a few inches in the soil.

Allocation of animals to experiments and animal variability

Cattle and dry sheep

Animal experiments are difficult to interpret and should not, therefore, be complicated by an unsuitable allocation of animals. Allocation is an intrinsic part of experimental design; the form of analysis adopted depends on design, so that faulty allocation may make valid analysis impossible. Crampton (1942) has pointed out that animal-husbandry experiments should be designed so that: (1) the imposed conditions to be measured statistically should operate without bias; (2) other factors known or suspected to be of importance to the results are subject to control or measurement either in allotment or by statistical procedures; (3) a valid estimate of the inherent and unaccounted-for variations between animals should be obtained. Experiments cannot be standardized beyond a few principles of procedure, but the precision obtained in them is in part determined by the number of degrees of freedom. The ideal is complete random allocation to treatment (Crampton, 1942; Finney, 1957) but because of limited land or numbers of animals, the experimentalist often has to balance his treatment groups in some way. Allocation to treatments must, however, be at random and there must be no changes once the allocation has been made. Groupings must be such that allowance for them can be made in the analysis, otherwise estimated error will be higher than if a purely random allocation has been made. Possible criteria for groupings are: previous treatment, weight, age, general appearance, type and sex. Groups should each contain at least as many animals as there are treatments, otherwise analysis becomes most complex. The main disadvantage of grouping is the loss of degrees of freedom for statistical analysis.

Farmers often complain that the results of small-scale experiments at research institutes may not be applicable in farming; and, from this point

*Figures in [] refer to Appendix 2.

of view, much can be said for using larger groups of unrelated animals. In using smaller groups, the aim is to reduce variability as much as possible, and related animals help to do so. Alder (1960) has surveyed the usefulness of monozygous and dizygous twins and half-sibs in animal experiments.

Ewes and lambs

There are special problems of allocation at lambing time, unless animals can be drawn from very large flocks. In general, lambs (with their ewes) may be introduced to an experiment either at the same age or at the same time. It is certainly easier to wait until all lambs are born and then allocate them to each treatment group. In many experiments, however, this is not satisfactory, since some lambs are exposed to a treatment at an earlier age than others and the ewes' milk supply may be differentially affected. All this may lead to unnecessary variability. In most recent grazing-season experiments at Hurley, in which ewes and lambs have been used, each ewe and her lambs have been introduced to the experiment within 24 hr of lambing.

Choice of animal and type of stock

Until recent years animal experiments were carried out with mature animals, either fattening sheep or cattle. As Watson (1948) has pointed out, this is not entirely satisfactory; these animals, when fattening, show a falling-off in their rate of increase; and, though it is well known that the nutrients required for each pound of increase rise as fattening proceeds, this is not the sole reason for dissatisfaction. Brown (1954) has emphasized that results obtained using sheep cannot be applied to cattle because of the different treading and grazing habits of these two classes of stock. Experience has shown that results obtained within a certain phase of an animal's life cannot be applied directly to other phases in the life of animals of the same species. For example, the sheep's life can be described in a number of phases: (1) the maintenance of the ewe before and during pregnancy; (2) the ewe with suckling lamb; (3) the lamb from weaning to fattening, or until old enough for breeding. Each phase may have an important bearing on the others: experiments should therefore study the whole of the animal's economic life, in addition to discrete studies of particular phases. With beef cattle the rearing period may have a profound effect on subsequent performance; the effect of store periods must be considered. For example, numerous experiments have shown a compensatory increase in rate of gain in a period of liberal feeding that follows a period of low nourishment and low live-weight gain both with cattle and sheep. Herbage intake studies have indicated that, where the available pasture is not limited, then intake of cattle previously fed on a low plane of nutrition is greater than intake of those previously fed on a high plane. This compensatory-growth effect and the difference in intake are of obvious importance when allocating cattle to summer-grazing experiments. The effects that store periods may have on subsequent performance and carcass composition must also be borne in mind when assessing results.

Animals may be used either (a) as a research tool or (b) as a product. When used as (a), some aspect of animal growth, performance or behaviour may be selected as the appropriate index. When used as (b), it is important to define the product and to employ measurements to indicate how many of the animals reach this standard in the various treatments or to measure the extent to which animals fall short. The choice of type to be used, therefore, must be governed by (a) that most appropriate to the investigation, or (b) that typical of the population to which the results are to be applied. Thus, in an experiment designed to study milk production of ewes, an entirely different ratio of singles to twins would be appropriate compared with that used in an experiment designed to study the effect of a husbandry system on lamb production per acre.

The use of 'worm-free' stock

The more or less complete absence of some or all of the nematode species parasitic in sheep or cattle is advantageous in experiments with nutritional or with parasitological objectives. The types of experiment in which 'worm-free' stock appear to be most useful are listed below:

Parasitological studies

The use of 'worm-free' controls reduces the need to distinguish between different levels of infestation. In experiments concerned with the effect of the parasite population on the host, 'worm-free' controls are desirable. The availability of animals free from worms allows the establishment of given levels of infestation with particular species.

The difficulty of maintaining animals free from these parasites over long periods is least in indoor experiments, where frequent cleaning of the pens and close control of food intake make it relatively easy. The difficulties must, however, depend on the extent to which an occasional parasite can be tolerated. In immunological investigations it is probably important that there should be no worms at all in the control animals: in many studies of the effect of worms on growth, it is unlikely that very small numbers (such as might remain undetected by faecal examination) would be of any significance.

At pasture these small numbers may be very important unless precautions are taken to prevent reinfection. Apart from the possibility of chemical procedures, there are two ways of achieving this. First, it is possible to fit animals with harnesses and faecal collection bags and thus prevent faeces ever reaching the ground. This method is unreliable and of very limited use. Second, it is possible to move the stock frequently on to clean pasture. In Britain, such pasture should have carried no ruminants for at least two years. The frequency of move may be based on the interval required for the infective larval stage of the parasite to be reached, an interval that may vary with temperature and other climatic conditions. Where these are variable it is better that the grazing period be too short for this development, whatever the weather. Accidental carriage of faeces on the feet of animals and similar considerations indicate that the more frequently stock are moved the better. The length of time that this can be

maintained is determined by the number and type of animals used, by whether supplementary feed is given and by the acreage of clean pasture available.

It will be noted that this rapid-folding technique has one major advantage; infected and non-infected animals can be grazed together, offered the same food and managed in the same way, without cross-infection from one group to the other. The chief disadvantages are: (a) comparisons with other managements cannot be made and (b) the situation is unnatural in that reinfection is ruled out. It is possible to overcome the latter restriction if 'worm-free' and infected animals are folded in adjacent paddocks.

Any other grazing management, although theoretically possible if the stock are completely free from worms, may break down at any time, since the accidental introduction of a small infection may rapidly result in normal worm-burdens.

Nutritional studies

The effect of any nutritional treatment on sheep or cattle at pasture is often obscured by the effects of worm-infestation. Nutritional effects may be exaggerated or prolonged, or even pass unnoticed, where parasitism is at a high level. Since there are interactions between parasitism and nutrition, and it may be desired to apply the results to normal (infected) stock, these experiments are ideally conducted with both worm-free and infected animals in each treatment group.

The difficulty in this approach is the same as that mentioned above. Either a rapid-folding management has to be employed, or completely worm-free animals should be grazed on clean pasture for as long as accidental infections can be excluded. Continuous folding over clean ground is appropriate to some investigations, particularly when comparing animal performance on pastures of a given composition or stage of growth. It is obviously unsuitable in any study involving regrowth of the pasture or different stocking rates. On the other hand, completely worm-free animals cannot be used in pasture experiments until they are old enough to be independent of other foods. If reared artificially they may be quite young (e.g. 6-weeks-old lambs); but their growth may then differ from that of suckling lambs.

A totally worm-free environment is clearly essential for flock studies or investigations into lactation and early lamb growth, in the absence of parasites. It is most appropriate for experiments on nutrition or management.

Where the interactions between nutrition and worm-infestation are important, both worm-free and infected groups are required. It is then difficult to prevent the one infecting the other, while ensuring that the pastures on offer to each group are comparable. It is here that the folding management is most useful.

Rearing problems

It will be apparent that the method of rearing has to satisfy criteria determined by the subsequent use of the animals: different methods are appropriate to different requirements.

Where the animals are to form part of a completely 'worm-free' environment one must eliminate all unnecessary risks of infection. It is then sensible to remove lambs from their ewes at birth and rear them artificially: with calves artificial rearing is in common use.

Artificial rearing

This can be done by any of the indoor methods described on pages 68-70. The only difference lies in precautions to prevent accidental infection. It is safer to prevent, as far as possible, any infection from entering the rearing house. Footbaths of disinfectant, steam-cleaning and washing of equipment can be effective against most species. In many experiments it is not necessary to exclude every species: *Strongyloides papillosus* and *Trichuris ovis* are often tolerated in small numbers because of the practical difficulties of excluding them. It is assumed that, in these experiments, their effect on the host is negligible.

Clearly the precautions taken must be related to considerations of this kind. It is, of course, safer to use such feeds as milk substitute and concentrates that entail no risk of infection. It is possible, but more difficult, to use conserved herbage or freshly cut material from uncontaminated fields, carted in such a manner as to preclude infection.

Rearing on the ewe

Where a very slight infection can be tolerated (or risked) the lambs can be left on the ewe. Infection of the lamb is then prevented by: (a) frequent cleaning of indoor pens, (b) rapid folding over clean pasture, or (c) some combination of indoor lambs with ewes brought in for suckling periodically during the day. It is sometimes possible to maintain lambs, reared in one of these ways, in a condition of apparent freedom from worms (*S. papillosus* is, however, usually present) for considerable periods (up to one year) at pasture without further folding. Sooner or later the few parasites which may have been introduced in only a few of the lambs begin to increase in number. If this increase is delayed by management or unsuitable climatic conditions, it is possible to carry out an experiment with virtually no interference from worms.

CHAPTER 9

METHODS OF ARTIFICIAL REARING

Calf rearing

The calf is used in two roles at the Institute. Firstly, it is employed as a test animal in experiments designed to study the effects of an introduction to a grass diet early in life. Secondly, it is exposed to the pre-treatment phase of some future experiment. In both cases, it is essential that groups of calves should receive the same basic rearing treatment and that data on the amount of food consumed during this period should be available.

The single-suckler method gives the highest animal performance but is least satisfactory from an experimental point of view. The intake of milk by the calf depends both on the vigour of the animal and on the milk production of the dam. Since calves reared in this way usually run with the cows at pasture and are free to suckle at will, it is impossible to assess the quantity of milk they consume.

The multiple-suckling method provides greater control of the calf and has the merit of producing well-grown animals. The calves are normally housed in groups of 2-4 and are suckled by a nurse cow at regular intervals. The quantity of milk consumed again depends on the vigour of the individual calf and the productivity of the cow. The intake of milk can be measured by weighing each calf immediately before and after each suckling. Care must be taken not to lose urine and faeces during the intervening period. It is possible, with a platform weigher [6] fitted with a dial scale reading in 1-lb divisions up to 800 lb and fitted with a suitable crate, to weigh animals to the nearest 0.25 lb. Trials extending over 12 weeks have shown marked between-milking and between-day variation in the intake of milk by groups and individual calves. There has been, however, a tendency for the 'rating' of calves within a group to remain constant. Although the calves soon learn the routine of moving on and off scales, the method is laborious. Furthermore, no indication of the quality of the milk consumed can be obtained.

The great advantage of the bucket-feeding system of rearing is that an accurate measurement can be made of the intake of milk by individual animals. The main disadvantages are the extra equipment and cleaning facilities required. In experiments where it is desirable to control the intake of nutrients, whole milk shows too wide a variation in quality. Where bucket feeding is employed, milk substitute, which is less variable, can be used. Commercial milk substitutes are not always suitable for experimental work because batches vary. Several milk substitutes have been used and have given satisfactory results. They include: (1) dried skim-milk powder; (2) dried butter-milk powder; (3) dried skim- or butter-milk powder, 4 parts, and finely ground oats, 1 part; (4) dried skim- or butter-milk powder, 4 parts, and glucose, 1 part. To each of these

four is added a vitamin supplement sufficient to provide each calf with 2000 I.U. vitamin A and 500 I.U. vitamin D per day.

The powders are offered at the rate of 1-1.5% of the live weight of the calf and are reconstituted by mixing 1 lb powder in 1 gallon warm water.

Faecal smears are taken for bacteriological examination and calves are kept in isolation on arrival. Each calf is dosed with aureomycin or other antibiotic should the examination indicate that this is desirable. Milk feeding is temporarily suspended and further doses given if white scour occurs. Milk or milk substitute may be offered to the calf in an open bucket, but the animal then tends to drink its ration too quickly; on several occasions this appears to have resulted in chronic bloat. The use of a system of feeding whereby each calf, by sucking a rubber teat, draws up the 'milk' through a plastic tube seems to have overcome this difficulty [14]. Calves may be fed individually from separate containers, or in groups from a common container. Under the latter system the calf's intake of milk depends on its vigour, so that it is not very satisfactory for experimental rearing.

The length of period during which milk or milk substitute is offered to the calf must depend to some extent on the experiment in question. A 12-week period provides the beef-type calf with a satisfactory start. It is possible, however, to wean the calf sooner; Preston (1957a) has suggested that 3-5 weeks may be a sufficient milk-feeding period. When early weaning is employed 'milk' must be replaced by dry food: and animals tend to be more variable in their intake of the latter. Where hay and concentrate feeding stuffs are provided, calves show individual preferences for these two feeds and some form of control must be exercised over consumption. If the quality of the hay is good, a satisfactory ratio is hay 2 parts (by weight) and concentrates 1 part. When hay is offered loose in a rack, it may be wasted, making it difficult to measure the amount consumed. If the calf is kept on a slatted or peat-moss floor it is possible to recover most of this wasted material, but this is not possible where straw is used as bedding. 'Chaffing' the hay into 1- to 2-in. lengths and offering it in a deep bowl reduces wastage and does not appear to affect its acceptability.

Recently Hibbs & Conrad (1958) have described the use of pellets in which ground hay and concentrate feeds were premixed in the desired ratio. The use of such pellets has obvious advantages although it is not clear what effect grinding has on the digestibility of the constituents. Concentrate feeding-stuffs are best offered to calves in a coarse form. Finely ground meals are not generally acceptable and losses may occur more easily. In order to measure the intake of these feeds it is necessary to weigh the amount offered, and that refused, over a 24-hour period. During the first few weeks of its life, the calf seems to have a widely fluctuating appetite for concentrate feeding-stuffs.

Preston (1957b) has calculated, by means of faecal-index techniques, the intake of herbage by grazing calves. The errors involved in these techniques, however, may be large. In order to obtain an accurate estimate of herbage intake, fodder cut from the pasture is used. Pre-weighed

quantities of a single cut of material may be cold-stored and used throughout a trial. This eliminates the need to make daily determinations of dry matter on offer, although individual refusals must be so analysed. A further advantage of the use of cut herbage is that it allows accurate assessment of the content of digestible nutrients. Calves find cold-stored herbage acceptable, but it is essential to change the material on offer at least once a day.

Artificial rearing of lambs

The possibilities of practical application of artificial methods vary from the rearing of orphan lambs to the rearing of lambs deliberately removed as surplus to the ewes' ability to suckle, combined perhaps with artificially induced prolificacy. Quite apart from the use of such techniques as part of a husbandry system, however, lambs successfully reared away from their ewes may have certain advantages as experimental animals. They can have a standard rearing period, which will largely eliminate the effects of single and multiple births and the difference in milk yield of the ewes. Furthermore, if the necessary precautions are taken, the lambs can be reared free from internal parasites (see p. 65 above).

Artificial rearing may continue for as long as a normal lamb would be suckled, or for a shorter period. The actual method used depends on the reason for rearing artificially and upon the numbers involved. For instance, in the case of the orphan lamb it would depend upon the age of the lamb when the ewe died or when it was removed. If the lamb is two or three weeks old it may be weaned on to a pelleted concentrate feed and eventually on to pasture alone. If only two or three lambs are involved in liquid feeding, a bottle is probably the easiest method or, if desired, the lambs may be trained fairly readily to drink from a bowl or trough. For larger numbers, more elaborate equipment is usually required, particularly where experimental animals are being reared.

Rearing may be carried out indoors or at pasture. Indoor rearing is desirable during bad weather at early lambing or out-of-season lambing (e.g. with Dorset Horns).^{*} It may also be necessary to rear the lambs indoors for experimental reasons, particularly where it is desirable to rear the lambs free from worms. Outdoor rearing may be more practicable, but some form of shelter from cold winds and rain is required. Rearing at pasture has the advantage of encouraging lambs to graze at an earlier age; this of course helps with early weaning. It is important to ensure that the pasture used is clean, e.g. not grazed by ruminants for two years; a newly sown ley is ideal. Observations have shown that the worm burden of artificially reared lambs can build up rapidly when they are grazed with normally infected sheep.

Liquid-milk feeding

Bottle feeding

This method is practicable only for a very small number of lambs, it being desirable to train the lambs to drink from a bowl or a bucket.

^{*}Unless the building is warm and dry, it is advisable to provide a source of heat such as an infra-red lamp.

Individual bucket feeding

It is necessary to bottle-feed lambs for the first day or two. They should then be encouraged to drink from a bucket, and this is readily accomplished by fitting a teat through a washer soldered into the bottom of the bucket (Plate 7). The lambs suck the milk through the teat and very little is left in the bottom of the bucket. This method has been successfully used at this Institute. Lambs are most easily managed in groups of six, a rack holding six buckets being used (Plate 7). This method ensures that each lamb receives its correct amount of feed.

Group feeding

This is carried out using a multiple suckler [14] (Plate 8) designed for rearing 12 lambs. Experience has shown, however, that a group of 6 is more easily managed. When starting lambs on the multiple suckler it is usual to place each tube into an individual container inside the main holder. As larger quantities are consumed, the small containers are removed and all the tubes are placed together inside the multiple suckler. One disadvantage of this method is that the stronger lambs will be able to take in more than their ration of milk. However, the multiple suckler may be the answer to the problem of feeding lambs in large numbers.

Artificial rearing of lambs has been carried out at this Institute with reasonable success using reconstituted full-cream dried milk. Ewes' milk is considerably richer than cows' milk, both in fat and solids-not-fat, and the milk powder was reconstituted at 1.5 times the normal strength (1.5 lb instead of 1 lb per gallon of water).

It should be ensured that lambs to be reared artificially receive a certain amount of colostrum. Experience indicates that two days old is the best age to remove lambs from their ewes; if they are left for longer it is much more difficult to train them for hand feeding. On removal they should be put into the rearing area and left for 12 hours or more to become really hungry before any attempt is made to bottle-feed them. In the case of orphaned lambs, which may be very weak, it is sometimes necessary to feed them forcibly and to keep them indoors near a source of heat for a day or two. Half a pint of milk-feed four times a day has proved a satisfactory regime but there are not yet enough data on the optimum frequency of feeding and the nutritional requirements of the artificially reared lamb. Much larger quantities can be fed successfully.

Experiments at Hurley have shown that although milk-feeding for 8 weeks gave slightly better growth than milk-feeding for only 5 weeks, weaning at the earlier age gave quite satisfactory results, provided that a pelleted concentrate feed was made available from the second week onwards. It would seem desirable to wean lambs by reducing the number of feeds gradually over a week or 10 days. It may be advantageous to add vitamins (vit. A at 100–200 I.U./lamb/day, and vit. D at 30–60 I.U./lamb/day) to the milk powder.

It is essential that the pelleted concentrate feed is introduced at an early age. The consumption of concentrates increases rapidly as the amount of milk feeding is cut down, so that as soon as the lambs are eating pellets readily it is possible to start weaning. Experience shows that

lambs will consume about 0.5 lb/head/day when 4 weeks old, and this will increase to 1 lb/head/day after weaning at 5 weeks. Concentrate feeding may be stopped at 6 weeks; and, after a slight check, lambs will continue to grow at a normal rate on grass alone. No check in growth rate is observed when milk feeding is cut down, as it is immediately compensated for by an increase in the consumption of concentrates. A temporary check may occur, however, when concentrates are withdrawn. Once lambs have settled down on an entirely grass feed they grow at approximately the same rate as twin lambs still suckling their ewes.

Lambs are provided with clean drinking water, and, if reared indoors, with good leafy hay (preferably cut from a 'worm-free' pasture). When handling milk and milk utensils, cleanliness is essential and all equipment must be sterilized regularly.

CHAPTER 10

MEASUREMENTS USED TO ASSESS ANIMAL GROWTH

Measurement of live-weight gain

Unfasted weights

Live-weight gain (per head or per acre) is used in experiments such as the comparison of pastures for feeding value or the comparison of grazing systems for parasite control or utilization of herbage. The duration of these experiments is governed partly by the object of the experiment but also by the rate of live-weight gain of the animal. In general, where live-weight gain is the primary index a young animal is used because an adequate (i.e. an easily measurable and statistically satisfactory) quantity of live-weight gain is achieved in a relatively short time. The final weight may be the main criterion of the product or (as in fat lamb) this may be better defined in qualitative terms (e.g. grading, cash value, carcass measurements). Where the live weight gained over the experimental period is the main result, the key weighings are obviously those measuring the initial and final weights of the animal.

A source of error and possible bias results from fluctuations in weight of 'fill' (the contents of stomachs and intestines). The weight of 'fill' in sheep and cattle varies with the quantity and quality of feed available, and with the time spent grazing or eating and drinking just before weighing. To keep these errors to a minimum, the day and time of experimental weighings are chosen in relation to the behaviour patterns of the stock and to the feed available.

Initial weights

In grazing experiments carried out over the summer season, the first weight is important, as it is used to calculate total live-weight gain and is sometimes used in allocating stock to treatments by weight (restricted randomization). Whether the stock are at pasture or in yards, they are kept in one group, or given feed from the same source in separate groups in order to provide uniform nutrition and 'fill' conditions. Two or three weighings, not necessarily on consecutive days, are recorded, and the 'initial' weight is an average of these.

Cattle wintered in yards are weighed before being turned out in spring in order to avoid the reduced 'fill' weight which has been shown by several workers to result from such a change of feed. Food is withheld until after weighing, which begins at approximately 9 a.m. In experiments where the final product from the animal is used the initial weight varies in importance according to whether other measurements are used to define the animal as it enters the experiment (e.g. newborn lamb, weaned lamb, over-wintered store) and what information there is as to the rate of growth up to that point.

Young calves and lambs may be weighed with a relatively high degree of accuracy since 'fill' is usually negligible. Birth weights of lambs are normally taken within 24 hours of birth, after the lamb has dried and before any appreciable suckling has occurred. A single weighing at birth suffices but initial weights, other than at birth, are based on weighings preferably taken on three successive days.

Intermediate weights

Intermediate weighings need not have the same accuracy since they merely show trends. The establishment of intermediate growth curves is important, however, if live-weight gain is to be expressed as gain per day or other short period or if there is any interest in when, and how rapidly, differences occur. Several factors determine the recording and interpretation of these weights.

Time of day. This may be important. For example, cattle weights recorded 3-4 hours after sunrise show less day-to-day variation than those taken later, and their level of variation is comparable with that obtained by fasting. This time is carefully observed in experiments studying 'fill'; in the majority of field experiments, however, where intermediate weights are used to indicate only the trend of differences in gain (and since herbage quality exerts an additional seasonal effect on 'fill'), weighing is carried out between 9 and 11 a.m. Weighing later is avoided because variability of grazing habit increases as the day proceeds.

Effect of grazing management. Management affects grazing behaviour, and consequently the weight of 'fill' at any given time of day, e.g. the mean live weight of a group of 2-year-old bullocks weighed daily has been observed to vary by 20-40 lb from one day to the next because of a change in availability of pasture when the animals were moved from one plot to another.

Under rotational grazing management, in which each plot is grazed for 5-10 days, the middle days are chosen for weighing. This avoids high and variable live weights which are encountered on the first two days, and variable weights on the last days on a plot.

Strip-grazing modifies the behaviour of cattle in the first 4-5 hours before the electric fence is moved each morning. Less time is spent grazing in this period and this leads to lower morning and higher evening weights than in rotational grazing, if the herbage is of comparable quality.

Frequency of weighing. Monthly intermediate weighings are often adequate for summer grazing experiments, but fortnightly weighing is practised for trials over shorter periods. When winter rations are to be adjusted to obtain a desired plane of nutrition, weekly weights are required. In certain critical short-period trials, indeed, stock are weighed daily for 5-6 days. Increasing the frequency of weighing, however, will not reduce the bias although with young calves and lambs it can give a valuable guide to performance because of the high proportion of the total weight that the gain represents.

Interpretation of intermediate unfasted weights. Because of the seasonal effect of herbage quality on unfasted weights, no interpretation of live-

weight gain in terms of body tissue increase and nutritive value of herbage is attempted within the season. Similar caution is observed in comparing treatments over parts of a season when herbage quality differences are marked. High weights are recorded on mornings of rainfall, due in part to wet coats and fleeces, but possibly also to a different rate of herbage intake during wet periods. This is not important in comparisons between treatments.

Final weighing

Where live weight is the main criterion this is determined on two or three successive days while, if the treatments are likely to produce marked differences in 'fill', the stock are grazed on a common paddock for two or three days before weighing. Alternatively, the empty weight is obtained at slaughter.

Fasted weights

Some advantage is to be gained as a result of the levelling effect of a 24-hours' fast without water on the residual 'fill' of cattle and sheep, but the practice may cause a reduction in live-weight gain. For investigations such as the *in vivo* estimation of body water using antipyrine (Brodie *et al.*, 1949), fasting for 24 hours has been used to reduce 'fill' differences between animals and between occasions. Weight of 'fill' at slaughter in individual steers aged 2-2.5 years has fallen within 9-14% of empty weight after 24 hours of fast without water. Although this represents a reduced variability (a range of 5 units) compared with the estimated 'fill' before fasting of 15-23% (a range of 8 units), individual differences in the region of 50 lb remain. These data suggest that 'fill' differences resulting from variations in quantity of intake may be more readily reduced by fasting than those resulting from differences in herbage quality.

Empty weights

These are obtained in the majority of experiments using fattening cattle and provide a most accurate final weight. Cattle are transported by lorry to the slaughter-house. They are weighed immediately before slaughter, and when hoisted after stunning, a nose-bag is fastened over the muzzle to prevent the loss of rumen contents by regurgitation. The oesophagus is tied when the head is removed, and the nose-bag contents weighed. The reticulo-rumen and abomasum contents are together emptied into a bath and weighed. The omasum is weighed full, and an average empty weight subtracted to give a close estimate of its content. The intestines are weighed before and after emptying. The total weight of contents of the stomach and intestines is subtracted from the slaughter-house live weight to give empty weight. A final weight for calculation of gain is obtained by adding a standard figure of 170 lb for 'fill' to the empty weight. This is an average figure for 'fill' in cattle slaughtered unfasted from the field, obtained from a number of experiments.

In sheep, empty weights are most valuable in the determination of dressing-out percentages.

Weighing equipment

For a number of years a livestock weigher [5] has been used for the individual weighing of cattle. This machine is not easily transported and so remains on one site throughout an experiment; otherwise it has proved most satisfactory. It has a capacity of 25 cwt, there are no loose weights and the steelyard is graduated in 1-lb divisions.

For calves and sheep a dial-type platform weighbridge [6] fitted with dials of different capacities and crates of different sizes has given good results. The design of these crates is important, from a practical point of view as it prevents livestock from turning round in the crate. These weighings are recorded to the nearest 0.5 lb. To enable accurate weighing to be carried out in a number of experiments, scattered over a considerable area, it is often convenient to use a mobile 'weighing trailer'. One used for sheep experiments at Hurley is illustrated in Plate 6.

Lambs are weighed in a canvas sling suspended from a spring balance (weighing to 0.1 lb), up to about 50 lb live weight.

Some additional measurements taken in ewe and lamb experiments

Wool. Measurements taken at Hurley have been largely confined to weighing the unwashed fleece, i.e. the year's wool growth. More detailed studies have been confined to simple measurements of staple length and weight of wool produced on a marked (10×10 cm) area of skin over a period of about 3 months. These methods have been based on techniques described by Lockhart (1954).

Udders. In studies of reproduction or nutrition during pregnancy and lactation, measurements of the udders may be used to indicate development of tissues associated with milk production. That most commonly employed is udder width (Thomson & Thomson, 1953). At Hurley the distance between the teats is measured external to them with a 12-in. pair of engineer's callipers, and read off against a metal ruler.

Milk production. Milk production in the ewe can be measured directly, by milking, or indirectly, through the lamb. The latter method may involve weighing the lamb before and after suckling (Owen, 1957), or may make use of the correlation between milk production and early growth of the lamb (Wallace, 1948). For comparative experiments on the milk production of ewes from treatments during lactation or in late pregnancy, the live-weight gain of the lambs from birth to 28 days is used, unless it is desired to know the absolute quantities of milk produced.

Measurement of growth and estimation of carcass growth in the live animal

Body measurements of animals are taken to indicate the effects of plane of nutrition upon skeletal and flesh development. Such measurements confirm whether live-weight changes reflect true carcass growth, as

distinct from differences in 'fill' due to treatment. Studies are also in progress to relate live-animal measurements in steers to carcass conformation.

The growth of the skeleton is normally correlated with live-weight increase and skeletal measurements are most useful where growth departs radically from the normal or where periods of maintenance or live-weight loss occur. The most useful are those which represent the growth of a selection of late and early developing parts of the animal.

Cattle

The main instrument used is shown in Fig. 7; it is based on the zoometrical rule described by Olbrycht (1945). This instrument consists of a sectional cylindrical brass rod marked off in 0.5-in. divisions. It is 72 in. long and includes a heavy screwed-on circular base-plate. The rod may be shortened to 48 in. by unscrewing one section. Two detachable arms, made of brass tube, are joined at right angles to the main rod. These arms are 12 in. in length from the main or vertical rod, and the length of each can be increased to 24 in. by screwing in extra sections. One side-arm is movable, sliding up or down the main rod, and may be secured by a pinching screw. Readings are taken on the main rod through a slit in the metal as indicated in Fig. 7. The second side-arm screws on at the base when the plate is removed and is so constructed that, in so doing, the length of the main rod is not altered. Some examples of the use of this instrument are given below:

<i>Measurement</i>	<i>Sections used</i>
Body length	Rod long+2 arms long
Height at withers	Rod long+1 arm long+base-plate
Height at elbow	Rod long+1 arm short+base-plate
Width at shoulder or chest	Rod short+2 arms long
Depth of chest or at navel	

The second instrument used for cattle is also shown in Fig. 7. This is a calliper which consists of a steel rule, marked up to 24 in. and 61 cm, embedded in a hardwood strip. Two sliding hardwood arms of 10 in. internal and 12.5 in. external length are set at right angles and are secured by pinching screws. This instrument is used to take width of hooks, length from hooks to pins, thurls width, leg thickness and leg width.

Finally, for girth measurements, a flexible, 4-metre, steel or plastic tape is used in conjunction with a small spring balance, to maintain a tension of 3 lb on the tape; or the steel tape may be used on its own for taking certain curved measurements.

Photographs taken against a squared grid can be used to show large treatment-effects on conformation.

Sheep

The following as used by Hamada (1950) are generally recorded (but only in experiments where this information is important): length of hind cannon; length of pelvis; width of hooks. All these are measured with engineer's (12-in.) callipers, readings being taken against a steel ruler.

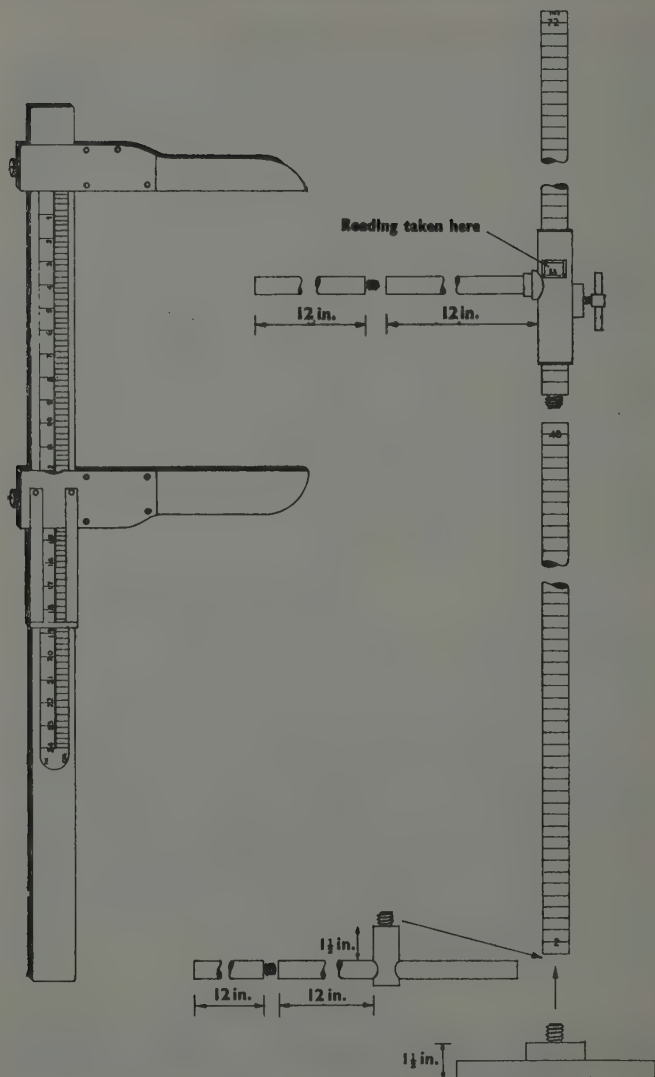


FIG. 7. Animal-measuring callipers and zoometrical rule.

With regard to frequency of measurement, the same considerations apply here as with weighing.

Carcass assessment

In some field experiments of a husbandry type it is often sufficient to record the total number or weight of the defined product (e.g. fat lamb). In others, grading of the carcass may be a necessary criterion of the satisfactory nature of the product. In more detailed growth studies, and particularly in studies where the nature of growth may be altered, it is advisable to consider the carcass produced in greater detail.

Estimation of carcass gain

The relationship between carcass weight and empty weight has been examined in over 200 cattle aged 2-2.75 years. It is found that the carcass weight increases, on average, by 73 lb with every 100 lb increase in empty weight. This figure is used to give an estimate of carcass gain per acre in season-long experiments, using initial and final weights.

A serial slaughter technique has been used to estimate carcass gains from pasture treatments, using store sheep and cattle. A representative sample of animals is withdrawn at intervals for the measurement of 'fill', carcass weight, and carcass quality. In one cattle experiment there were three slaughter dates at intervals of three weeks, covering the period of grazing on spring pasture immediately after the end of the winter-feed period. Carcass gains for the remaining cattle were calculated by estimating their carcass weight from those of the slaughter sample. This assumes that the dressing-out percentage of the slaughter sample (i.e. the carcass weight expressed as a percentage of the live weight in the field) is representative of that of the remaining cattle, a procedure used in studies of carcass wastage during travel (Callow, 1955, 1957). This technique is capable of extension to longer grazing periods and allows the estimation of gain in empty weight and carcass weight in different parts of the season and in different seasons.

Estimation of carcass composition

(a) *To provide a measure of utilized energy production from pasture from an estimate of carcass gain.* In terms of energy, values of live-weight gain and carcass gain are not necessarily comparable. Callow (1948) showed that the calorific value of boneless meat is directly related to the percentage of fatty tissue in the carcass. Data from the dissection of steers of 1-2 years and of 2.75-4 years were used to calculate a theoretical partition of the components of carcass gain during fattening for each group (Callow, 1949). Making an assumption that 70% of live-weight gain is carcass gain, the live-weight increase in young and old steers that would give an equal increment in fatty tissue has been calculated from these partition coefficients. This shows that boneless meat of equal calorific value would be obtained from a live-weight gain of 300 lb in old steers and 522 lb in young steers. Even higher rates of gain in growing lambs and calves would not necessarily produce a higher calorific value. This is

because of the low fat-content of the tissues in animals at a young stage of growth and the high proportion of protein, which is associated in muscular tissue with three times its weight of water (Callow, 1950).

(b) *To measure carcass value per se.* In the production of the meat animal from pasture, high live-weight gains per acre can be obtained by carrying high stocking rates, possibly at the expense of reduced live-weight gains per head. Low gains per head will be reflected in the proportions of fatty and muscular tissues laid down in the carcass. The measurement of these proportions, which affect the value of the carcass, thus becomes an integral part of the assessment of any pasture treatment in which the meat animal is subjected to varying levels of nutrition.

Determination of slaughter date

There are four main approaches to the determination of date of slaughter when carcass composition is to be estimated.

(a) *Stock from different treatments are slaughtered on the same date.* Comparison at the same date is used, e.g. in hormone-treatment trials where the effective period of absorption of the implanted hormone is fixed; in some fat lamb experiments; and in serial slaughter trials for evaluating gains over a fixed period, where date of slaughter *per se* is to be studied in relation to herbage quality and 'fill' differences existing at a given time. This method, in which both carcass weight and its composition contribute to differences in the value of the product, gives more information than (b), (c) or (d) below. It avoids the complications involved in the varying length of time needed to bring stock to constant carcass weight or fatness. These complications include seasonal variations; e.g., in herbage conditions, or in worm burden in lambs. Methods (b), (c) and (d) may, however, approach commercial conditions more closely, and may thus be more suited to comparisons involving pasture management systems *in toto*.

(b) *Slaughter at equal fatness.* In some experiments particularly with fat lambs, stock are slaughtered when they reach a constant state of 'finish' or percentage fatty tissue in the carcass. This subjective grading is difficult in practice. No rapid and accurate objective method of assessing fatness in the live animal has yet been perfected. Such is the influence of fatness on carcass quality (Callow, 1947, 1948) and on the public taste in beef (Pomeroy, 1956) that this method is desirable when suitable assessment can be achieved. It is in occasional use at this Institute.

(c) *Stock from different treatments are slaughtered at equal live weights and, if possible, equal carcass weights.* Measurements of carcass composition are used to determine whether, and how far, the end-product has been affected in its proportions of bone, muscle and fat. The time taken to reach equal carcass weight then becomes an important measure of treatment difference. This method is used in trials to evaluate winter feeds for store animals, when the time needed to reach equal carcass weight on summer grass reflects the extent of winter depression of live-weight increase and subsequent recovery.

(d) *Slaughter when treatment feed supply ends.* In cattle experiments the date of slaughter has been most often determined by the exhaustion of available supplies of feed in each treatment. Carcass value is then determined not only by weight and composition, but also by price per pound within the seasonal price structure for cattle and sheep. Date of slaughter again becomes an important measure of treatment difference. This method is aimed at an evaluation of the pasture management as a whole, carried out in a manner suited to application in commercial practice.

Methods for assessing carcass composition

In store lambs and fattening sheep, use has been made of published regression equations (Pálsson, 1939) that relate carcass measurements to the weight of dissected tissues in the whole carcass. With Suffolk $\times$ Half-bred lambs good agreement has been found between actual carcass weights and those obtained by addition of calculated weights of bone, muscle and fat. With hoggets, however, total calculated tissues fall short of the actual carcass weight by an average of about 8 lb, with individual carcasses differing by as much as 25 lb. Since the latter were the longest, it is probable that our hoggets grew to a greater overall size than Pálsson's. His measurement of carcass length, which was incorporated in other equations, could not readily be obtained in commercially handled carcasses. This is an instance of the possibility that serious bias may emerge when relationships established in one population of animals are used to calculate carcass composition in another. Even within the same breed, differences in type and varying planes of nutrition may affect the relationships when they are applied to other animals.

In cattle, fatty tissue, muscular tissue, and bone in the carcass are estimated by dissection of a loin or, with larger numbers, of the 13th rib. Relationships between the percentage of each tissue in these joints and in the whole carcass for Herefords, Shorthorns and Friesian and for mixed breeds have been obtained by Callow (1958). The process of dissection, weighing, mincing and sub-sampling was found to require approximately 7 man-hours per rib and 14 man-hours per loin. A small test of specific gravity measurement as an estimate of fatness of the rib joint emphasized the need to standardize storage time, and showed a high error of prediction.

Measurement of carcass quality

This includes the methods for estimating carcass composition described above, but embraces other measurements which describe quality in other ways.

Subjective grading based on conformation and finish

This routine is carried out by graders of the Ministry of Agriculture, Fisheries and Food on carcasses from experiments. Every effort has been made to supplement their assessment by more objective measurements of the same aspects of quality.

Linear measurements

These are intended not only to describe the size and shape of the skeletal framework but also to measure the fleshing of the carcass at

different points of varying value, i.e. its conformation. For sheep, Pálsson's system (1939) has been applied as completely as commercial handling of the carcasses allows: for cattle, measurements developed at Cambridge have been used. Experience of these has been incorporated in recommendations by the Agricultural Research Council Study Group on Beef Carcass Quality (1959). When dealing with relatively large numbers of carcasses at the slaughter-house (up to 16 sides at a time), simplicity of operation is essential. Thus, the minimum amount of apparatus is required—a steel tape and wooden callipers (see Fig. 7) are found to be sufficient separate items to handle. The simple measurements are therefore preferred to more complex systems.

The measurement of conformation by weight

This is feasible in certain experiments. The weights of joints have been recorded at the time of cutting in London and Home Counties style (Gerrard, 1951; Harrington & Pomeroy, 1959). This provides a basis for the examination and comparison of linear measurements for use when jointing is not possible. The cutting must be kept constant throughout the experiment and measurements from reference points introduced where possible. An estimate of carcass composition, using a sample joint, should also be included.

Meat quality

In certain experiments, samples of muscular tissue are taken in order to assess meat quality. Samples have been preserved in formalin for the subsequent measurement of muscle fibre diameter (Joubert, 1956). Routine methods of chemical analysis are used for the determination of fat and moisture in minced tissues from sample joints. Further means of assessing factors such as tenderness are needed.

CHAPTER 11

OTHER MEASUREMENTS

Animal behaviour studies

A knowledge of the behaviour of grazing animals is important to a complete understanding of productive performance. Behaviour studies are particularly valuable in the study of management systems, of palatability, and of the natural conditions of climate and environment. They can also be of value to relate investigations of food intake and faecal output. Tribe (1950) and Hancock (1953) have reviewed much of the work on them.

In work on herd behaviour the number of animals engaged in the various activities being studied is usually counted at short intervals (usually 2 or 4 minutes) throughout 24 hours, or shorter periods. In studies of individual animal behaviour it is possible to record continuously, or if several individuals are being observed, by making recordings on each at set intervals. The equipment required for this type of study consists of a shelter for the observer, binoculars, a torch and a stop-watch. The techniques used are similar to those described by Johnstone-Wallace & Kennedy (1944) and Hughes & Reid (1951). Canaway *et al.* (1955) developed a simple apparatus for the continuous non-subjective recording of cattle movement and activities under field conditions. This apparatus has not been used in general management studies, partly because it would not be possible to mix the bullocks being so observed with others, and partly because analysis of the paper records is time-consuming.

Parasitological techniques*

Both lungworms and gastro-intestinal worms may cause considerable damage to the host; and parasitological techniques have been primarily concerned with assessing their importance. These techniques may be used in experiments designed to study reciprocal animal or pasture growth. A measure of any variation in parasite populations resulting from variation in management is a useful adjunct to other measurements taken in interpreting the pattern of growth of the host animal. Numbers of adult worms can be assessed adequately only *post mortem*, but representative samples of the intermediate stages of the parasites may be readily collected; and they provide some measure of the infection established or to which the animal is exposed. The accuracy of such assessment depends largely on: the technique used, the frequency of sampling, the size of the parasite population.

*The authors wish to acknowledge the considerable help received from the staff of the Ministry of Agriculture, Fisheries and Food, Veterinary Laboratory, Weybridge, Surrey, in connection with these techniques.

The population may vary considerably with the management to which the host is subjected, with the location and meteorological conditions and with the capacity of the parasite to enter and become established in the host. Thus studies in the field, even under controlled conditions, cannot ensure the maintenance of a constant parasite population.

Daily sampling may be necessary in purely parasitological studies (and experiments such as short-duration digestibility trials): less frequent sampling may be adequate in experiments from which only a generalized curve of infection over a long period of time is required. The shorter period of investigation requires more frequent sampling; and it may be unprofitable to exceed sampling intervals of one month in the long-term experiment.

The technique used is related to the size of the parasite population present. For example, at high levels a sub-sample count of the number of eggs in the original faecal sample (using the McMaster slide) is probably adequate, whereas at lower levels a more sensitive technique is needed.

Sampling techniques

Sampling techniques are employed both in the field and in the laboratory and are concerned with the collection of faeces and their examination for the presence of eggs of gastro-intestinal worms and 1st-stage lungworm larvae, or with the collection of grass and its examination for the presence of 3rd-stage infective larvae of both parasites.

Field techniques

Faeces collection. Eggs of gastro-intestinal worms and larvae of viviparous lungworms are passed out of the host in faeces, which thus provides a useful means of assessing the degree of infection present. Faecal samples can be removed directly *per rectum* but in field experiments at Hurley samples are always taken after normal defaecation. Each animal concerned is carefully watched until it defaecates, when a sample of approx. 10 g (sheep) or 20 g (cattle) is collected by means of a clean spatula and put in a wax carton. The animal must be clearly identified, preferably by a number painted on the rump or flank. Two practiced workers can sample 50 sheep per hour.

Grass collection. Larvae of both types of worm may be found on the grass in the third or infective stage of development. The grass therefore provides a second source for assessing the population, viz. that to which the host is likely to be exposed. Methods of grass collection described by Taylor (1939) and by Michel & Parfitt (1955) are used in a modified form. Duplicate samples, each of approx. 8 oz, are collected in plastic bags. The operator follows two predetermined W-shaped routes across the plot in such a manner that the two routes intersect and cover the whole area to be sampled.

Since the amount of grass on a field may be either sparse or plentiful it follows that the number of sub-samples taken (at every third step) must vary between fields.

Crofton's work (1954) on the migration of infective larvae has suggested that higher numbers of larvae are to be found on the grass immediately before and after noon, whilst low numbers are recovered under very wet conditions. It is the practice therefore at the Institute to collect grass samples at approx. 11 a.m. on days when rain is not actually falling.

Laboratory techniques

The essence of the method involved in the recovery of these intermediate stages of the parasites is to separate them from the faeces or grass. Parfitt (1955 and 1958) has summarized the techniques commonly used for this purpose, and the following (i) and (iii) represent modifications of these techniques.

(i) *Faecal egg separation.* For recovery of helminth eggs from sheep faeces, 1 g of the individual or mixed bulked sample is weighed, transferred to a 50-ml flask and well mixed with approx. 7–8 ml distilled water, using a glass rod. The mixture is next passed through a sieve (80 meshes to the linear inch) into a 15-ml centrifuge tube by way of a glass funnel. The sieve contents are sprayed with just sufficient distilled water to fill the centrifuge tube. The faecal suspension is then centrifuged for 2 minutes at 1000 r.p.m., after which the supernatant liquid is quickly decanted leaving behind a sediment of faecal residue containing the helminth eggs. Saturated zinc sulphate solution (sp. gr. 1.180) is then added almost to the brim of the centrifuge tube and the mixture is stirred with a thin wire. A coverslip is moved over the mouth of the tube, sufficient zinc sulphate solution being added from a dropper to prevent the formation of air bubbles beneath the coverslip as it is moved to seal the mouth of the tube. The mixture is centrifuged again for 2 minutes at 1000 r.p.m. in order to allow the unwanted faecal material to settle; the high sp. gr. of the solution prevents sedimentation of the eggs. Finally, the coverslip is removed with a clean, upward lift and placed over a microscope slide ready for further examination (iv).

A similar process is used for recovery of eggs from faeces of cattle, but larger samples of faeces are generally used.

Larger samples are also collected from animals either reared 'worm-free' or having only a very light infection, since small infections are not readily detected in small amounts of faeces.

(ii) *Faecal larvae separation.* The method is used for the detection of the larvae of *Dictyocaulus viviparus* in cattle faeces. It differs in the following ways from the method described in (i): 10 g of the faecal sample are weighed out. After sieving, the filtrate (which has a relatively large volume) is transferred to a 1-pint measure and allowed to settle for 3 hours. The supernatant liquid is siphoned off and the residue is then ready for the addition of the solution of high specific gravity. The saturated solution used in this case is common salt, which has been shown to give more consistent results than saturated zinc sulphate solution (Parfitt, 1958).

(iii) *Separation of larvae from herbage.* The method is similar to that described by Parfitt (1955) and is used for the recovery of infective larvae of both lungworms and gastro-intestinal worms.

A 0.5-lb sub-sample of the collected grass, or the weighed quantity if less, is placed in an enamel bucket to which 2 gal of tap-water are added. After soaking for 2 hours the contents are stirred and the grass is removed in small amounts, after being squeezed. It is then placed in a second bucket and water is again added. After a further 2 hours the grass is removed in a similar manner and discarded. The two buckets are allowed to stand overnight while the contents settle. The larvae which are active migrate from the grass and fall towards the bottom of the bucket. The next morning the supernatant liquid is siphoned from each bucket, leaving behind about one pint. The sides of each bucket are carefully sprayed with water and the contents are then passed through an 80-mesh-per-in. sieve into pint measures and allowed to settle for 2 hours. After the supernatant liquid has again been siphoned off, the residue is ready for the addition of saturated solution of common salt: the technique then followed is similar to that described in (i) and (ii).

(iv) *Faecal-egg-counting technique.* The temporary mount prepared by the technique described in (i) is examined systematically under the low power (2/3 in.) objective of a microscope equipped with a mechanical stage. All eggs identified as belonging to the orders of parasitic nematodes are counted. Separation of the count is generally made into: (1) eggs of the family Trichostrongylidae (excluding *Nematodirus* spp.) and the sub-family Oesophagostominae grouped together, (2) *Nematodirus* spp. and (3) *Trichuris ovis*. Eggs of *Strongyloides* spp. and oocysts of coccidia are not normally counted. Although it is preferable to complete the egg count soon after preparation, it has been found possible to store slides in a refrigerator at -4°C for periods up to a week without serious deterioration (Spedding, 1952).

If faecal-egg-count data are to be used to indicate the size of the adult parasite population present, they must be related to a number of factors, including component species which differ in egg-laying capacity. The egg count may be extremely variable both within one day (Spedding, 1952) and from day to day (Spedding, 1953). It cannot, therefore, be used as a precise index of the size of the worm population: it is more precise as a measure of the number of eggs being deposited on the pasture. It is of most value as an index of the level of worm infestation where sufficient animals are sampled frequently over a considerable period. Egg-count data give no indication of the larval population carried by the host and it should be remembered that damage can occur while faecal-egg counts remain relatively low.

(v) *Larvae-counting technique.* The temporary mount prepared by the technique described in (i) and (ii) is examined in a similar manner to that in (iv). Where faecal examination for lungworm larvae is made, any larvae present will be in the 1st (non-infective) stage of development, whereas in herbage examinations larvae of lungworms and gastro-intestinal worms will usually be in the 3rd (infective) stage of development. Identification may

be limited to groups (such as *Nematodirus* spp. and the Trichostrongyle group [including Oesophagostominae]) or every individual may be identified separately. The latter may require many detailed measurements depending on the experience of the operator. Larval preparations cannot be so readily stored as are faecal-egg preparations and it has been found advisable to complete the larval count as soon as possible after preparation.

(vi) *Culture methods.* A broad classification of the component species is derived from the faecal-egg count, although it is possible to differentiate species more fully by the method described by Cunliffe & Crofton (1953). Specific differentiation is more readily made by culture methods. Sufficient faeces (about 50 g) are placed in a glass jar to a depth of approx. 1 in., and a few drops of distilled water are added, as required, to ensure a moist environment. The lid is then loosely screwed on. Too much faecal material prevents the emergence of larvae in the lower regions. The sample is incubated for 3 to 5 days at 29°C. During this time sufficient larvae may develop to provide a representative proportion of the larval population present in the faecal sample. If all the larvae capable of development are to be identified or counted, a longer incubation may be necessary. At the end of this period larvae may be observed on the sides of the jar below the faeces level (in air pockets) and also above it. An upward migrational response is evident if the larvae are exposed to a stimulus of diffuse light for approx. 0.5 hour. Any larvae adhering to the sides of the jar may then be washed out by lightly spraying the sides of the jar above the faeces level with distilled water before carefully pouring the liquid into a centrifuge tube. The larvae may then be concentrated into a small amount of liquid by centrifuging. A small drop of the culture is placed on a microscope slide and the larvae fixed in an elongated position, either by adding a drop of iodine solution or by gently heating the slide on the microscope lamp. A coverslip is placed over the preparation which may then be examined. Larval measurements are taken by means of a micrometer eyepiece and specific identification is made by reference to a classified key, such as that prepared by Dickmans & Andrews (1933).

(vii) *Post-mortem worm counts.* In experiments which terminate in the slaughter of the experimental animals, the number and species of adult roundworms inhabiting the alimentary tract of the fat lamb or the numbers of larvae or adult lungworms in the lungs of cattle may be assessed.

(a) *Gastro-intestinal worms.* The whole of the alimentary tract between the oesophagus and the rectum is removed and the tissue knotted at each end to prevent leakage. The material is transferred in covered buckets from slaughter-house to laboratory, where the oesophagus, rumen, reticulum, omasum and rectum are dissected and discarded. The remaining material is then divided into three, viz. the abomasum, small intestine and large intestine (colon and caecum together), each of which carry different species of worms. Each part is washed with tap water, the abomasum and large intestine being cut open for this purpose, and a few ml of formalin are added. The washings from each region are passed through a coarse

sieve, washed repeatedly with tap water, and the larger worm species picked out with forceps. At least 150 ml are sub-sampled from the filtrate and examined for microscopic worms.

(b) *Lungworms*. The lungs are removed from the thorax severing the trachea close to the larynx. The entire bronchial tree is then dissected with a pair of scissors, starting at the common trachea and proceeding down to the finer branches in each lung. The larger worms are picked out and then the lung is thoroughly washed in normal saline solution to recover immature worms. A further extraction process (mincing the lung tissue and the use of a Baermann technique) may be used to recover any larval stages present. This method is similar to that described by Michel (1954).

The measurement of herbage digestibility

The digestibility of any component in a feed is defined as the percentage of that component which, when eaten by the ruminant, is not excreted in the faeces*. The percentage of the organic matter in herbage which is digested is at present the most useful measure of the feeding value of that herbage because, while it cannot be as precise a measurement as net energy content, it is so much more readily determined that it can be applied to a study of a very large number of herbage feed variables (species, variety, maturity, season, etc.). In addition, herbage digestibility data are used in estimations of herbage intake in the field (p. 93).

The digestibility of herbage organic matter may be calculated from the relationship:

$$\begin{aligned} \text{\% Digestibility} &= \frac{\text{Wt. O.M. eaten} - \text{Wt. O.M. in faeces}}{\text{Wt. O.M. eaten}} \times 100 \quad \dots \quad (1) \\ \text{of herbage O.M.} & \end{aligned}$$

(O.M. = organic matter)

In a digestion experiment the herbage under test is fed to experimental animals for a preliminary period in order to adjust the animals to the feed and to establish a steady rate of passage of feed through the tract. This is then followed by a trial period during which feed intake and faeces production are measured. A 'preliminary' period of 7 days appears adequate, unless the experiment involves an abrupt change in type or level of feeding from that previously given. The 'trial' period should be as long as possible, and preferably of 10–12 days duration. The supply of a uniform herbage feed for the total period of up to 21 days poses a problem not found with earlier feeding experiments, which dealt mainly with concentrates and dry roughage feeds. Thus, fresh herbage cut daily will change markedly in maturity and composition over this period, so that no precise definition of the 'herbage fed' is possible. Various workers have overcome this by cutting herbage on one day and artificially drying it before feeding it in a digestion trial. At this Institute cold-storage (see Chapter 24) is used to preserve herbage cut on one day for feeding over the whole of a digestibility experiment lasting 3 weeks. This technique markedly reduces labour,

* In all cases faeces include endogenous and exogenous excretions, so that 'apparent', and not 'true' digestibility, is measured.

compared with daily cutting of herbage for feeding, and permits herbage cut at one season to be fed at any other time of year, so allowing the most efficient use of equipment. It has an advantage over dehydration since the herbage is still fed as a 'fresh' feed, which has not suffered the physical and chemical changes likely during heat-drying. However in certain cases (p. 97) digestibility data on herbage cut daily may be of value.

As noted above, a study of herbage feeds requires a large number of digestibility experiments and the facilities described below were planned for this requirement. However, the techniques and much of the equipment used are equally applicable to experiments with a smaller number of herbages. Sheep are used as experimental animals, because they require only one-sixth the amount of fresh herbage needed by cattle. Where it is essential that cattle be used, equipment described elsewhere (Balch *et al.*, 1951) will be necessary.

Animal house

A single large room, $55 \times 25 \times 13$ ft, is used to house 32 sheep cages. This is brick-built and has a flat concrete-slab roof. There are windows in the east wall and a 'lantern light' in the roof, so that the sheep are not in direct sunlight. The floor is of slightly roughened concrete with a fall from the centre line to side gutters. The sheep cages are arranged in two lines, with a central feeding passage (Plate 9).

Sheep cages

These are made of timber (for cheapness and warmth), are open-sided and are wide enough to allow the sheep some movement and a view of its neighbours. These factors help to maintain appetite and well-being. The cages are designed for use with male sheep, the faeces being collected in bags carried by the sheep, (p. 88). Urine is collected in trays, also carried by the sheep (p. 88), or, when collection is not required, it is allowed to fall through the floor grid on to a sloping metal sheet. The back of the cage is completely open to allow ease of handling, and each sheep is held in its cage by a chain and spring clip fastened to a collar (Plate 11). The herbage is offered in a feed bin hooked to the front of the cage and large enough to minimize loss of feed through it dropping on the floor. Each cage has a separate water container. All woodwork is painted with a grey lead-free paint [15] and the cages are steam-cleaned every 6 months and repainted where required (Plate 10).

Sheep harness

The sheep are fitted with leather harness (to which the faeces collection bag is attached), which is also used when weighing the sheep [17]. A separate collar is used for holding the sheep in the cage. This harness (Plate 11) has also been used successfully for collecting faeces from free-grazing sheep.

Faeces-collection bags

The canvas bags generally used in digestion trials are not suitable for collecting the very wet faeces voided by herbage-fed sheep. Rubberized canvas bags, built round a shaped metal insert at the mouth, are therefore

used [12]. They are held in position by 4 cords attached to the harness and fitted with quick-release clips. They do not collapse when the sheep lies down, the wire insert can be shaped to fit the particular animal used, and they are readily emptied and washed. These bags have been used for total faecal collections in numerous grazing experiments in which as much as 5 kg of fresh faeces have been collected in 24 hours from a single sheep, the bags being emptied twice during the day.

Urine collection and nitrogen balance

In experiments in which the nitrogen balance of an experimental animal is being studied, total collection of urine during days 10 to 22 is carried out by using a metal tray suspended under the sheep's belly by cords. The urine runs from the tray through a wide-bore rubber tube (at least 0.5-in. bore, to avoid blockage by wool). It is collected in a 1-gal polythene bottle from which it is transferred each morning to a 5-gal container kept at 5°F in the cold-store. At the end of the experiment the total collection of urine is thawed, weighed, mixed thoroughly, and a sample taken for analysis for nitrogen, energy (p. 147), etc., all these being carried out on a weight-of-urine basis. This system has considerable advantages over the usual method of daily weighing and bulking of aliquots.

Cold-storage of herbage feeds

A digestibility experiment is generally carried out with 3 sheep, each fed for 21 days. From 16 to 20 lb fresh herbage, containing 2–3 lb D.M., are fed daily, giving a requirement of approximately 1200 lb of fresh herbage per feed tested. The amount of herbage needed is cut in the field and brought as rapidly as possible to a covered bay. 'Long' herbage is cut into 1-in. lengths with a power-driven chaff-cutter before mixing and weighing, but short-cut herbage can be mixed immediately. After thorough mixing, fresh herbage is weighed into tared hessian sacks, each sack containing exactly the required weight, usually 18 lb; larger weights of herbage per sack become increasingly difficult to freeze. To speed weighing all sacks are weighed individually at the beginning of the season and divided into 'batches' at 0.1-lb intervals. All the sacks used for each lot of herbage are from one 'batch', and so have a fixed tare. The sacks of grass are weighed on a spring balance, graduated to 0.1 lb, with this tare allowance made [10].

For a 3-sheep digestion trial, 60 sacks of herbage are generally weighed. At the same time 12×300 -g samples of herbage are taken, dried at 100°C, and reweighed to find the dry-matter percentage in the herbage. From this the average dry-matter content in the herbage in each sack is calculated. The dried herbage samples are then combined and milled (p. 139) for subsequent chemical analysis. A representative sample of herbage is also taken and kept in cold-storage in a polythene bag for botanical analysis, leaf/stem separations, etc., as required.

The weighed sacks are tied at the mouth with labelled strings, and one sack placed on each layer of a stacking trolley. These are designed with trays so as to allow loose grass to be frozen if required; a simpler

design can be used if grass is to be frozen in sacks. Each trolley, when loaded with 8 sacks, is wheeled into the 'blast-freezer' room in the cold-store block (p. 150). Inside this room the air is circulated very rapidly over cooling coils, through distributing ducts into the room and round the sacks of herbage, and then back over the coils. The room is cooled to -10°F before loading and will then freeze $200 \times 18\text{-lb}$ sacks of grass (approx. 1.5 tons) in the following 30 hours. Where possible the herbage is left in the blast-freezer for 48 hours to ensure complete freezing at the centre of each sack; the frozen sacks are then transferred and stacked in the main store at 5°F . The stacks are built on spaced railway sleepers and iron rods placed at intervals in each stack to help conduct away any pockets of heat. These precautions are important, for herbage is very difficult to freeze. During the early stages of cooling the herbage continues to respire and the heat of respiration is added to the normal freezing load of the wet herbage. Also, conduction of heat from the grass in the centre of the sack is slow, so that adequate time must be allowed for it to freeze completely.

The feeding of frozen herbage

A sack of the appropriate frozen herbage is removed from the cold store at 9 a.m. each morning for feeding to each sheep. A portion of the herbage is put into the feeding bin and can safely be fed without prior thawing. Sheep have been fed without ill-effect for up to 12 weeks on such herbage: it seems likely that the digestive disturbances reported when animals eat frozen feeds result from their ingesting rotten or contaminated materials which they do not distinguish when frozen, but which they would reject under normal conditions. Further feeds are given at 11 a.m., 2 p.m. and 5 p.m., the herbage left in the sack remaining partly frozen and so in fresh condition. Much of the herbage, of course, thaws in the feed bin before it is eaten.

Experimental sheep

In earlier work (Raymond *et al.*, 1954b) it was shown that the digestive efficiency of sheep increases by approximately 1% per year as the animals grow older. It is now recognized that this largely results from the effects of gastro-intestinal parasites (Spedding, 1954): as sheep become older they become increasingly immune to these parasites and their depressing effect on digestive efficiency decreases. For the most precise studies worm-free sheep (p. 64) appear necessary. In current studies sheep aged 15–27 months are used. These have developed considerable resistance to parasites, but at the same time are still growing rapidly enough to allow useful measurements of live-weight gain and nitrogen retention to be made. They are dosed at 2-monthly intervals with 10 g of phenothiazine to reduce further the effects of parasites. While no difference in the digestive efficiencies of sheep of the same age, but of different breeds, has been demonstrated, it was decided in all these studies to use sheep of the same breeding, namely Suffolk $\times$ Half-bred. Castrated sheep born in the farm flock in February/March of one year are used in digestion experiments in May in the following year and for a period of 12 months.

In general, the sheep remain in cages during two consecutive digestion trials (6 weeks) and then spend 3 weeks in the field. Under this regimen they keep remarkably healthy, though it is necessary to pare and trim their hooves at intervals. In cages the harness must not fit too tightly and the animals should not be shorn too closely, as a very short fleece encourages harness sores. A shearing head is used which leaves a good cover of fleece. The animals must be crutch-clipped frequently to keep them clean.

Experimental procedure and weighing

All sheep are weighed at the beginning and end of each experimental period and also at intervals during the experiment. This is done by lifting them by their harness with an electric chain hoist [4] from which a spring balance is suspended (Plate 9). The hoist is supported on a bogey on an overhead railway, which allows it to be moved from cage to cage. In this way one operator can weigh the 32 sheep in 25 minutes.

During the preliminary period each sheep is fed daily one sack of the herbage being examined. Where this rate of feeding is found to be excessive, two sacks of herbage may be fed every 3 days. At 9 a.m. on day 8 all feed residues are removed from the feed-bin, floor, cage, etc., and the experimental period starts. It continues until 9 a.m. on day 20. During this period the number of sacks fed (and so the weight of dry matter offered) is recorded. Feed residues are collected every one or two days and a complete collection of residues from feed bin, floor and cage is made on day 20. All these residues are dried at 100°C, weighed and the total weight subtracted from the feed offered to give feed consumed. Where selection of feed occurs, rate of feeding is decreased to reduce the degree of selection. The residues are dried, milled and analysed chemically. The results are used to estimate the composition of the feed eaten.

At 9.30 a.m. on day 10 the faeces bags are emptied, and total faeces collection is made from then until 9.30 a.m. on day 22. The time lag of 48 hours spreads the work at the beginning and end of the experiments: where the level of feed intake varies during the experiment it makes some allowance for the time of passage of the feed through the digestive tract, faeces production on days 10 to 22 being assumed to result from feed consumed on days 8 to 20. Where only short collection periods (e.g. 4–6 days) are used, this assumption may introduce considerable errors because of end-errors resulting from non-uniform passage of feed through the tract (Raymond *et al.*, 1953).

The faeces from each sheep are collected in a 5-gal tub fitted with a lid. The bags are emptied each morning and the tubs are then placed in the cold store at 5°F and brought out next morning for a further collection. Where very large quantities of faeces are being produced it may be necessary to have two collections daily, at 9.30 a.m. and 4 p.m. In addition, a small tin is hooked behind each cage and into this any faeces spilt from the bag are placed: these faeces are not cold-stored.

On day 22, each faeces bag is emptied into the appropriate tub and the tubs are then allowed to stand until the faeces have thawed (generally in about 48 hours). The tub (previously tared) plus faeces are then weighed

to obtain the weight of fresh faeces. The faeces are then tipped into the bowl of an electric mixer and thoroughly mixed. The machine used [13], has a capacity of up to 80 lb of fresh faeces. From the mix 3×500 -g samples of faeces are accurately weighed into trays, dried for 24 hours in a drying oven (p. 135) and then reweighed. Any spilt faeces are also dried and weighed and the total faecal production is then calculated. Where an electric mixer is not available the faeces should be mixed carefully with a spade on a metal sheet: it is then advisable to take 5×500 -g samples to allow for the rather greater sampling error possible with this method of mixing. After weighing, the oven-dried faeces are milled (p. 139) prior to chemical analysis.

At the same time as faeces samples are taken for dry-matter determination, a further 3-kg sample of faeces is placed in an airtight tin and returned to the cold-store. This is used for analyses on 'fresh' faeces as required, e.g. for chromogen and nitrogen.

Digestibility experiments with herbage cut daily

Herbage cut daily will change in composition and digestibility during a digestibility trial and the use of dehydrated or frozen herbage is therefore advocated for the precise measurement of the digestibility of herbage at a given stage of growth (p. 86). However, in connexion with techniques for estimating herbage intake, digestibility data on herbage harvested daily during the experimental period are of real use as they allow a 'local faecal index regression', relating to the sward being studied, to be derived (see p. 96 and Raymond *et al.*, 1956a and Corbett & Greenhalgh, 1960).

In this case sufficient herbage, cut each morning with a power mower, is brought to the animal house and thoroughly mixed. In order to ensure that the same weight of herbage dry matter is fed each day, a rapid dry-matter determination is immediately carried out by placing 2×100 -g samples of the fresh herbage, thinly spread in large trays, into a preheated drying oven (p. 135). Drying is generally finished in under one hour, and the average weight of the two samples gives the approximate dry-matter percentage in the herbage, from which the fresh weight of herbage to be fed is calculated. This weight is then weighed out for each experimental sheep, three 400-g sub-samples being taken at the same time for accurate dry-matter determination. The conduct of the digestibility experiment is then exactly as described above for frozen herbage, except that faeces are weighed and sampled for dry matter daily and aliquots bulked for chemical analysis every 4 days.

Calculation of results

The percentage digestibility of the dry matter in a herbage (D) is calculated thus:

$$D = \frac{\text{(Wt. of D.M. in herbage eaten)} - \text{(Wt. of D.M. in faeces voided)}}{\text{(Wt. of D.M. in herbage eaten)}} \times 100 \dots (2)$$

The digestibility (per cent) of any other component is calculated thus:

$$\text{Digestibility of component } (D') = \left[\frac{100(Y-X) + XD}{Y} \right] \dots \dots (3)$$

where Y = percentage component in feed,
and X = percentage component in faeces.

To eliminate errors due to soil contamination in the herbage fed, digestibility results for herbage are most usefully reported on an organic-matter basis. This requires determination of the ash content of the herbage fed, A_Y , and of the resulting faeces, A_X . In equation (3), Y is then $(100 - A_Y)$ and X is $(100 - A_X)$, D' then being the percentage digestibility of the organic matter in the herbage.

Labour requirements

As the number of sheep fed in digestion trials at any time increases, the labour needed per animal decreases with the wider spread of 'overhead' work. The present 32-sheep unit occupies only 2.5 man-hours daily, except at the beginning and end of each 3-week feeding period. Weighing, mixing and sampling the faeces from 32 sheep takes approximately 8 man-hours and weighing and milling the dried faeces at the end of each experiment a further 8 man-hours. In addition, approximately 7 man-hours are required to put in the cold-store each 0.5-ton batch of herbage delivered to the weighing bay.

Estimation of the herbage intake of grazing animals

While properly conducted grazing experiments will allow valid comparisons of management systems, sward types, etc. in terms of animal production, they can seldom provide satisfactory explanations for the differences found. These result largely from the different nutrient intakes of the animals grazing the different treatments, and estimates of the nutrient intake of grazing stock would thus be of great value in the interpretation of these experiments. Two methods of investigating nutrient intake are being studied at Hurley, as well as at many other centres, based on herbage-cutting techniques and on the study of the faecal output of the grazing stock.

A. Herbage-cutting methods

The deficiencies in present herbage-cutting techniques for estimating grazing intake have already been discussed (p. 41). However, they have a further limitation when *nutrient* intake is being estimated, in that the latter is the product of two estimates:

wt. of herbage consumed (lb) $\times$ nutritive value per lb of herbage.

Nutritive value has been predicted from the chemical composition of the herbage grazed (in turn derived from the yields of 'in' and 'out' cuts and their chemical compositions, and subject therefore to accumulated error) but it is clear that the errors in this prediction, at least with the

information available to date, are considerably greater than is generally realized (see Minson & Brown, 1959). This error, added to the error likely in the estimate of herbage intake from cutting data, make this method of little use for estimating any but the largest of differences in nutrient intake between pastures.

B. Faecal techniques

These have derived from the technique for estimating grazing intake, originally proposed by Garrigus (1934). This uses a re-arrangement of equation (1), (p. 86):

$$\text{Wt. herbage organic matter eaten} = 100 \times \frac{F}{100 - D'} \dots (4)$$

where F = weight of faecal organic matter
and D' = digestibility of organic matter eaten.

It further allows an estimate of nutrient intake, in terms of digestible organic matter:

$$\text{Digestible organic matter intake} = 100 \times \frac{F}{100 - D'} \times D' \dots (5)$$

if both the faecal production of grazing stock, and the digestibility of the herbage they consume, can be measured.

Faecal production

In many experiments faecal production has been measured by equipping the grazing animals with harness and bags, so allowing total faecal collection. However, this method is laborious, the harness may occasionally worry the animals and lack of return of faeces to the sward may interfere with long-term soil-fertility experiments. An alternative technique is based on the use of indigestible external tracers, first proposed by Edin (1926). A known weight of the tracer is fed daily to each animal and it is then assumed that the same weight of tracer is excreted in the faeces. If a truly representative sample of the faecal excretion is obtained and analysed for its content of tracer, total faecal production can then be calculated thus:

$$\text{Faecal production} = \frac{\text{Wt. of tracer fed}}{\text{Wt. tracer per lb of faecal organic matter}} \dots (6)$$

(lb organic matter)

The most widely used tracer is chromic oxide (Cr_2O_3), which is cheap, inert and non-toxic, and has been shown to be effectively indigestible. To give a suitable concentration of Cr_2O_3 in the faeces, sheep are generally fed 2 g, and cattle 20 g, per day. The oxide can be fed mixed with a concentrate (as is generally done with dairy cattle), given as a drench in bentonite suspension (Raymond & Minson, 1955) or given in gelatin capsules*. When fed in capsules, the oxide can be weighed into 2-piece gelatin capsules, but it is more convenient to use ready-filled capsules [11]. These

*The use of kraft paper impregnated with chromic oxide has recently been reported by Corbett (1959).

are available in 1-g and 10-g doses. The chromic oxide is in maize-oil suspension in the capsules, which are coloured orange to aid detection of occasional capsules which may be rejected by animals after dosing. The capsules are administered by a balling gun, that for sheep being purchased and that for cattle being made in the Institute workshops (Plate 12). The determination of the chromic-oxide content of faeces is described in Chapter 25.

The major problem with such an 'external' tracer is that it does not mix uniformly with the feed eaten and is thus not mixed uniformly in the excreted faeces. A diurnal pattern of faecal concentration results, with the same number of peaks as the number of doses of tracer given. This pattern was described by Edin (1926) and Kane *et al.*, (1952), but insufficient attention has frequently been paid to the error it may introduce in faecal estimations. In particular it has been assumed that the excretion pattern is constant, so that representative faeces may be collected at fixed times when the faecal excretions contain the true mean concentration of chromic oxide. However it has been shown (Raymond & Minson, 1955) that the diurnal pattern may in fact vary markedly with type of feed and level of feeding and that the assumption of a constant excretion pattern by grazing stock is untenable.

Alternative techniques have been developed in which a representative sample of the faeces produced is obtained by systematic sampling of the faeces on the sward.

Sheep. A number of fixed pegs are located in a stratified randomization on the sward. Each morning a loop at the end of a 4.5-ft wire is placed in turn over each peg, and all the faeces lying within the 'ring' described around the peg by the end of the wire are collected. The faecal collections from all the 'rings' on the sward are combined; these will be faeces produced during the preceding 24 hours, as all the 'rings' will have been cleared on the previous day. The exact number of 'rings' required must be decided in the light of: (a) the area of sward, (b) the number of sheep, (c) the frequency of dosing, and so the extent of the diurnal fluctuation in faecal chromic oxide and (d) the precision desired. In our own experiments 20 rings used on an area of 0.25 acre grazed by 6 mature sheep have given a C.V. of 7% in the estimated concentration of Cr_2O_3 in the faeces excreted on one day, when Cr_2O_3 was given twice daily.

Cattle. With cattle, sampling from each 'pat' of faeces produced has been adopted. The investigator walks systematically over the pasture, collecting a large spoonful (20 g) of faeces from each fresh pat of faeces. After sampling, each pat is sprinkled with chalk so that it will not be sampled again on a later occasion. The combined spoonfuls are thoroughly mixed to give a representative sample of faeces for analysis for chromic oxide content (p. 153) and Faecal Index components (p. 95).

Frequency of dosing chromic oxide

It has been clearly shown (Pigden & Brisson, 1956) that the range of the diurnal variation of Cr_2O_3 concentration in faeces is reduced by frequent dosing during the 24 hours. However, this is generally impractical in

grazing experiments. It seems preferable to adopt once- or twice-daily dosing (with the resulting large diurnal variations in Cr_2O_3 excretion) and undertake the more intensive sampling of faeces from the sward which is then required to obtain a representative sample of faeces.

Identification of faeces from individual animals or groups of animals

The above techniques have the disadvantage, compared with the direct rectal sampling usually adopted, that they give only a combined sample for all the animals grazing the sward. With cattle this has been overcome by dosing each animal with gelatin capsules containing coloured polystyrene particles, each animal being given a particular colour (Minson *et al.*, 1960). Some 70% of these particles are excreted undamaged in the faeces, where they can quite easily be distinguished, so that each pat of faeces on the sward can be allocated to the particular animal by which it was voided. Enough polystyrene must be given as a dose to allow easy identification of the faeces: with young stock a daily dose of one capsule containing 18 g of polystyrene is generally adequate, but with 1000-lb bullocks two capsules are necessary. Polystyrene, like chromic oxide, must be fed for several days before the start of the experiment, so as to distribute it throughout the digestive tract. This technique has proved particularly useful when groups of cattle from different treatments (e.g. winter managements) are put under uniform treatment on one sward. In this case all the cattle in each group are dosed with particles of the same colour, allowing samples of faeces from each group to be collected separately.

These particles are however not suited for use with sheep, which grind them so finely that they cannot readily be identified. The coloured particles [16], in 5 colours, are available in 1-, 2-, and 5-kg packs, together with 2-piece gelatin capsules for dosing.

A similar technique has been developed by Line & Head (1960) at the National Institute for Research in Dairying, Shinfield.

Digestibility of herbage grazed

The calculation of nutrient intake from equation (5) requires also an estimate of the digestibility of the herbage organic matter grazed. This differs from the digestibility of the herbage on offer because grazing livestock tend to select from that herbage the more digestible components, grass- and legume-leaf in preference to stem. It is not possible to harvest 'herbage as grazed' for determination of its digestibility in an indoor feeding experiment and for this reason an indirect technique, the Faecal Index method, has been widely adopted. In this, data on cut herbage fed indoors are used to calculate a relationship of the type shown in equation (7) between the digestibility coefficients of the dry matter or organic matter in herbage feeds, and various chemical constituents in the resulting faeces:

$$\text{Digestibility of herbage organic matter} = a + bx \dots \dots (7)$$

(where x = concentration of chemical component in the faeces)

By measuring the concentration of the particular chemical component (x) in the faeces produced by grazing stock and substituting in equation (7), an estimate of the digestibility of 'herbage grazed' is obtained.

Equations of this type have been derived in which herbage digestibility is positively correlated with the percentage nitrogen content (Lancaster, 1949) and the pigment concentration ('chromogen'), (Reid *et al.*, 1952) in faeces, and negatively correlated with the percentage normal-acid fibre content (Raymond *et al.*, 1956b).

This technique has been widely used in grazing experiments, but too little attention has generally been paid to the errors likely in its application in the field. Thus, herbages tested have often not been within the 'population' of feeds used to establish the published relationships: in many experiments no account has been taken of the prediction errors associated with the Faecal Index regression equation used and conclusions have been drawn about differences between herbages which are quite non-significant. Further, it is not certain how far these relationships, based on data from stock fed indoors, are valid when applied to grazing stock.

The majority of published relationships of the form (7) have had standard errors greater than ± 3 digestibility units, which means that no significance can be attached to predicted differences between the digestibilities of grazed herbages of less than 9 digestibility units. Because of the form of equation (5), in which $(100-D')$ is used, the corresponding errors in intake estimates will be considerably larger. Thus an estimate of digestibility of 75 ± 3 units will result in a prediction error of $\pm 17\%$ in estimated intake from equation (7) which means that estimates of nutrient intake differing by less than 50% are unlikely to be significant. To be of real use in experimental work a Faecal Index equation should have a prediction error of less than ± 1 digestibility unit, allowing differences in digestibility between feeds greater than 3 units to be detected. Even at this level, differences in nutrient intake of less than 15% are unlikely to be significant.

At present it seems that error limits as low as ± 1 unit can only be attained by basing a regression equation on a very restricted range of feeds. Such an equation is then, however, of little use to other workers, because of the uncertainty as to whether the herbage being investigated is within the population on which the equation was based. Because of this, it seems necessary to advise that all grazing-intake experiments should be accompanied by digestion trials in which herbage similar to that being grazed is cut and fed indoors to animals similar to those being used in the field study. The method then becomes similar to that originally used by Garrigus (1934), except that allowance is made for selective grazing by the use of a modified Faecal Index technique. This can be done by the use of a 'local' regression or of a 'correction' method.

'Local' regression method. This is based on digestibility/faecal composition data from indoor digestion trials carried out on herbage cut from areas similar to those on which herbage intake is being measured. Herbage is generally cut daily for feeding indoors (see p. 91) and herbage digestibility calculated on 4- or 6-day sub-periods. A 'local' regression equation is then calculated from these digestibility figures and from the data for faecal composition during the corresponding sub-periods. Table 1 gives an

example of this method, based on data from herbage cut daily during three 4-day sub-periods.

Table 1.

Derivation of 'local' faecal index regression using digestibility data (4-day sub-periods) from fresh herbage cut daily

Cut	% herbage O.M. digested	% N in faecal O.M.
A. 11/5-14/5	72.9	4.10
B. 15/5-18/5	71.3	3.55
C. 19/5-22/5	68.8	3.10

Regression equation (best fit):

$$\% \text{ herbage organic matter digested} = 56.6 + 4.04 \times \% \text{ N in faecal O.M.}$$

Thus if % N in faecal O.M. from grazing stock = 4.25%, estimated digestibility of herbage grazed = 73.8%.

The main limitation of this system is that it does not provide enough data to give error estimates and this may be particularly serious where there is marked selective grazing by stock, when the faecal N from these stock will be well outside the range covered by the indoor feeding experiments. It is also important that the herbage for feeding indoors should be cut at the same time of year as the grazing experiment is conducted, because of the large 'season' component in faecal nitrogen regressions (see also Corbett, 1960).

'Correction' method. In this the slope of the Faecal Index regression for a particular sward is assumed to be the same as that of a published relationship, but the constant of the regression is determined by a single indoor digestibility experiment on herbage as similar as possible to that being grazed.

An example of the use of this technique follows. Published regression equation (Raymond *et al.*, 1954a):

$$\% \text{ herbage organic matter digested} = 44.8 + 7.95 \times \% \text{ N in ash-free faeces.}$$

From the indoor experiment it was found that the digestibility of the cut herbage was 75% and the nitrogen content in the faecal organic matter was 3.5%. Thus the 'corrected' regression relating to the sward under study was:

$$\% \text{ herbage organic matter digested} = 47.2 + 7.95 \times \% \text{ N in ash-free faeces.}$$

The 'correction' method makes the assumption that the regression coefficient of a published equation applies to the particular sward being studied. This will rarely be true, but the error introduced will be small if the herbage being selected by the grazing stock does not differ too markedly from that cut for indoor feeding.

It cannot be emphasized too strongly that the use of a published Faecal Index equation without carrying out any digestion experiment to check whether it is applicable to the particular sward being studied is likely to give invalid results.

Application errors

As noted above, errors may also arise from the application of an equation based on indoor feeding experiments to stock grazing in the field. One source of error should be eliminated whenever possible by using the same type of animals in the indoor and outdoor experiments. This is particularly important with faecal nitrogen relationships, because of the marked effect of internal parasites in sheep on faecal nitrogen excretion. If sheep must be used in indoor digestion experiments associated with cattle-grazing experiments, mature sheep, which are relatively immune to internal parasites, should be used. 'Chromogen' excretion does not appear to be so affected by such parasites and because of this may be a more valuable Faecal Index component than nitrogen.

An error also seems likely because of the difference in levels of intake between grazing and stall-fed animals (Raymond *et al.*, 1959). It has been shown (Raymond *et al.*, 1956a) that at a high level of intake herbage is less efficiently digested than at a low level, but that differences in faecal composition do not fully allow for this. In the case of fibrous constituents in faeces (which are negatively correlated with herbage digestibility) animals on a high level of intake give a lower fibre percentage in the faeces than animals fed on the same feed at a low level, implying in fact a higher digestibility at the high level: this makes faecal-fibre/digestibility relationships quite useless for predicting the digestibility of grazed herbage.

Both faecal nitrogen and faecal chromogen appear partly to compensate for differences in level of feed intake. In the case of faecal nitrogen, however, it has been calculated from results of 'level-of-intake' experiments that indoor data are likely to overestimate digestibility in the field by some 1-2 units (Raymond *et al.*, 1959). Because of this it seems reasonable to subtract 1.5 digestibility units from such estimates of the digestibility of grazed herbage, to allow for 'level-of-intake' effects. It is hoped that further study will allow a similar correction to be made to estimates based on faecal chromogen. It is obvious that these corrections cannot be precise, because the differences in intake level between field and indoor conditions vary so markedly under different conditions, but to make some correction for the 'level-of-intake' effect seems sounder than to take no account of it.

If every effort is made to reduce prediction errors and the effects of level of intake, the two methods described here may possibly allow differences in nutrient intake by stock on different swards to be detected if they are greater than about 15%. Evidence on the errors in these techniques suggests that, no matter how carefully the experiments are conducted, estimates of differences between grazing intakes less than this are unlikely to be significant and unless greater differences are expected there seems little point in employing these techniques.

PART IV

PLANT/SOIL STUDIES

CHAPTER 12

THE CONDUCT OF LEY AGRONOMY EXPERIMENTS

These experiments, at Hurley, investigate the influence of ley management on the yield of crops taken after the leys have been ploughed. Variation in management of the leys is widely interpreted to include differences in species composition of the leys, various patterns of use of the herbage grown and fertilizer practices on the ley. Cultivation practices during the conversion of leys to tillage cropping are also investigated. In order to elucidate the causes of differential yields following variable ley management it is frequently desirable to introduce differential treatments during the test-cropping phase.

Size of plots

In experiments involving yield determinations of crops during the ley and tillage phases the plot size or shape adopted will be that which best suits all crops in the sequence. To some extent the size and shape of the plot will be a compromise between those desirable for each crop taken during the course of the experiment. The type of management imposed on the ley (e.g. cutting or grazing), ploughing, cultivation and harvesting procedures will all have a bearing on determining the convenient and practicable plot size and shape.

Plot size for the ley phase

Where grazing treatments are involved it is necessary to have all grazing-treatment plots individually fenced in order to confine the influence of each treatment to the area on which it is practised. Loss or gain of fertility arising from unequal distribution of animal excreta is minimized by individual grazing management of treatment plots separately fenced.

Although long and narrow plots are most efficient in the majority of field experiments, they involve certain disadvantages for grazing purposes. Groups of grazing animals tend to congregate towards the ends of long narrow plots and these areas receive an undue proportion of the dung and urine voided. For these and associated reasons the plot shape adopted is a rectangle in which the ratio of width to length is 1 : 1.5.

The area of grazing plots is arranged so as to hold a small group of animals for a minimum period of two days in order that the excreta voided will be related to the herbage produced on the plot. The minimum number of sheep in a group which has proved successful in experiments

is 4 or 5; this number behaves and grazes normally and the individuality of any single member of a group is not excessively expressed.

At Hurley, where total annual dry-matter yields of about 4000 lb to 9000 lb per acre per annum are available for grazing, the actual grazing-plot size adopted is 16×10 yd (1/30th acre approx.). Each plot is fenced and is grazed by 4–8 sheep for two or three days. The concentration of a larger number of sheep in a plot of this size, even though there may be ample forage for them, is not generally successful, since a large proportion of the herbage is liable to be trampled and rendered unpalatable soon after the animals are introduced.

When the herbage in the ley phase is subject to cutting treatments, the plot size could be reduced. The minimum in these circumstances would be that which could be successfully harvested when in the arable cropping phase. However, the majority of experiments include both grazing and cutting treatments and the plot size adopted has been that necessary for the grazing treatment.

Transfer of fertility

The individual grazing of plots will largely retain fertility arising from stock excreta within those plots. However in- and out-going animals may add or take away fertility to an extent that depends on the nature of the herbage they have been grazing before being introduced to the treatment plots and on the difference in quantity and quality of gut fill at the time of entry or of leaving the plots. In order to avoid this type of fertility transfer one of two procedures has been used, depending on circumstances. Where treatment plots in all replicates are grazed simultaneously the procedure has been to introduce animals which for the previous 24 hr have all been on the same grazing and on herbage as similar as possible to that of the treatment plots. If fertility is brought on to the treatment plots it will then produce a uniform bias over all plots in relation to their stock-carrying capacity.

The alternative is to provide an additional replicate of treatment plots (pre-entry plots) which are grazed first and the animals then transferred to the same treatments within the experiment proper. The grazing is then continued from replicate to replicate: the groups of animals are kept to their appropriate treatments.

Plot size for the arable crop phase

The following observations apply to the cereal crops (wheat, barley and oats) and to kale. The cereals are normally harvested by a self-propelled combine [20]*, modified as described below (p. 112).

Plots of 7×3 yd, yielding as little as 12 lb grain, have been harvested satisfactorily. Plots suitable for individual grazing by sheep (16×10 yd) are thus capable of being sub-divided in the test-cropping phase. They are frequently split lengthwise to give two sub-plots of 16×5 yd: they have been split to give up to 5 plots each of 10×3.2 yd.

Yields of kale are obtained from plots of 16×10 yd or 16×5 yd by cutting samples within the plot.

*Figures in [] refer to Appendix 2.

CHAPTER 13

ESTIMATION OF WEIGHT OF ROOTS AND UNDERGROUND ORGANS IN GRASSLAND

The method and equipment used for estimating the weight of underground organs in grassland soils in common use at the Institute have been described (Williams & Baker, 1957). The procedure has not changed since then, except that the core sampler is now driven into the ground mechanically.

Sampling tool

This is a cylindrical sampler of 3-in. internal diameter at the cutting edge and 2 ft long. The internal diameter of the tube is increased by 0.25 in. from a point about 2.5 in. from the cutting edge to facilitate the withdrawal of the soil core. The cutting edge is tipped with a hard steel alloy. At the striking end the sampler is strengthened by an external collar welded on to the outside. A circular striking cap of soft iron is made to fit over this end (Plate 21).

In moist, stone-free soils, it is possible to drive the sampler to a depth of 12 in. fairly easily with a sledge hammer. When the weather is dry, or the ground stony, sampling becomes harder and distortion and breakage of samplers occur. Under these conditions a mechanical post driver [23] has greatly eased and increased the speed of the work. The post driver is mounted on the hydraulic lift of a tractor and operated by the power take-off (Plate 21). The sampler is also lifted out of the ground by the hydraulic lift.

With this equipment two persons can take 18–24 samples in an hour.

The storage of soil samples for later separation of roots

Storage by immersion in 4% formaldehyde (using a horticultural grade of formalin) or at low temperature (-15°C) has been equally successful. Samples stored under the latter conditions are thawed in a tank of formaldehyde solution before washing the roots free from soil.

Washing the roots

A root-washing apparatus is used which washes the bulk of the soil off the roots. Final separation of root and other underground plant organs and debris from coarse sand and stones is done by decantation. The mixture of roots, rhizomes, stolons and plant debris is separated by hand into the various categories as required by the investigation.

Experience has shown that, even after careful washing, the root material contains a considerable and variable proportion of soil. For this reason the root material (or a representative ground sample) is ignited in a muffle furnace at 600°C and the loss on ignition is taken as the weight of ash-free organic matter in the sample.

Sample variation

The weight of ash-free organic matter in the roots extracted from individual samples is highly variable and coefficients of variation from 20 to 35% have been recorded. In order to estimate the weight of root material under grassland swards intensive sampling and adequate replication are required.

CHAPTER 14

THE MEASUREMENT OF THE EFFECT OF LEYS ON SOILS

Because of the considerable length of rotations, which include leys of three or four years' duration, and the fact that, in experiments, the effects of treatments and cropping sequences must be examined over a number of seasons, it may be necessary to follow changes in the soil for several decades. Again, these changes may be small relative to the condition of the soil under arable cultivation. For these reasons, the soil is analysed when it is taken from the field and again later for comparison with samples obtained from the same plots at later stages in the rotation.

The basis for selection of analytical techniques

Different systems of agriculture affect many characteristics of arable soils. Of the major soil components, organic matter is perhaps the one most influenced by agricultural practices. An initial selection of other features for analysis was based on two criteria: (a) that changes should be estimated in the physical condition and in the nutrient status of soils under leys; (b) analytical techniques should permit an examination of representative soil samples from a large number of field plots and should give results in a form suitable for statistical evaluation.

The following are the properties of the soil examined as a routine practice in the laboratory:

- (i) Total organic carbon,
- (ii) Total nitrogen,
- (iii) Soil incubation characteristics
 - (a) The mineralization of nitrogen,
 - (b) The evolution of carbon dioxide,
- (iv) Water-stable aggregation.

Arising from the requirements of these analyses, the moisture content of the soil at 75 cm water tension, and pH are also determined. The carbon present in separates of macro-organic matter (e.g. root weights) is estimated for a wide range of ley treatments.

Field sampling

A number of problems arises in sampling soil from field plots under rotations which include a grassland phase of several years' duration. The tufted nature of many grasses, variation in the clover content and the very local distribution of faeces and urine introduce sources of variation greater than those present in soils under annual tillage. Any restriction on randomization must be predetermined and clearly defined (i.e. stratification of sampling regions in a pattern over the plots and the discarding of fence-line borders). At some stage of decomposition, faecal

patches are difficult to define by the operator in the field. An attempt to avoid any faecal patches is therefore considered undesirable, although their inclusion will increase sample variability.

Methods of soil sampling should not damage large areas of sward: this largely precludes digging pits in small experimental plots. It is desirable that samples should be related to any profile variation under the several phases of the rotation. Both the degree of soil compaction and the marked profile development in organic matter and other soil characteristics under 3-year leys must be considered in relation to sampling depth. In the case of land newly sown to leys the initial sampling is delayed until 6 months after sowing and thus allows for some compaction of the ploughed horizon. After a few years under grass, sampling depth must be closely controlled if analyses are to be comparable since considerable differentiation will have occurred within the top soil profile.

Of the many tools which have been used to obtain soil for analysis, sampling tubes are recommended as they can be rapidly filled and emptied. They also reduce contamination and ensure adequate control of the volume of soil contributed to the sample from the different parts of the profile. A sampling tube for taking cores of about 1 in. diameter to plough depth is shown in Fig. 8. This design has been found satisfactory, even when moderate amounts of stone are present, for all but the heaviest of soil. To minimize compaction, the sampling head (Fig. 8B) is turned with a slight internal taper. A sharp cutting edge is required to ensure that the

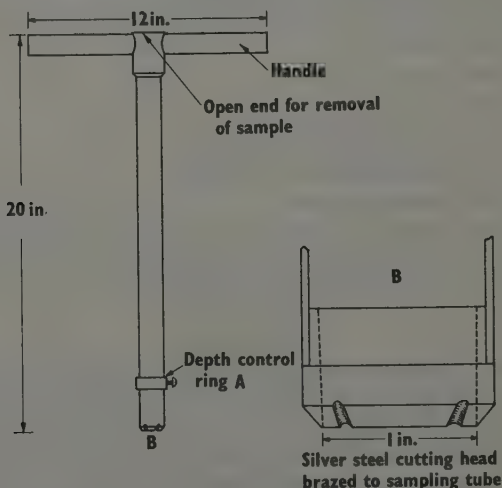


FIG. 8. Soil-sampling tube (mild steel handle and collar welded to stainless steel tube ext. diam. $1\frac{1}{4}$ in., int. diam. $1\frac{1}{8}$ in.).

turf is cut and that roots and underground plant material are properly sampled. A notched edge, used with a twisting to-and-fro action to cut into the soil, was found to be more effective than a smooth cutting edge. A depth-control ring (Fig. 8A) determines the depth of the sample. With a twisting action, and with only moderate pressure, the sampling tube will cut through the turf and into the soil until the depth ring makes contact with the soil surface. A final complete turn, before removing the tube from the soil, shears the base of the core. With the sampling tube inverted, only slight pressure is necessary to eject the core into an aluminium tray or other suitable container.

Thirty cores of 1 in. diameter to 6 in. depth are usually bulked to form a plot sample. Randomization over the area of the plot (commonly about 160 sq. yd) is restricted by taking three zig-zag traverses, each of 10 cores. The bulked sample is passed through a 0.5-in. square-mesh sieve. Stones exceeding this size, herbage stubble growing above soil level and clover stolons are discarded. Dead leaf litter and all plant material below the surface of the soil are cut so as to pass through the screen and are retained in the sample.

For a plot sample consisting of ten 1-in.-diameter cores, the standard deviation of a plot estimate of soil organic carbon (with a mean value of 1.33%) was $\pm 0.062\%$. With 30 cores per plot the standard deviation per single bulked plot sample may be calculated as $\sqrt{\frac{0.062}{3}} = 0.036\%$ organic carbon: a coefficient of variation of 2.7%.

The storage of soil samples

The conditions of soil storage must inhibit, or greatly reduce, biological and chemical activity. After storage, the soil should be in a condition which may be readily brought to the physical form and moisture content required for any particular method of analysis. Two methods of soil storage are in use:

Storage of dry soil at room temperature. Soils are dried at 30°C in a fan-ventilated oven. The soil is spread in a thin layer in shallow trays to minimize any incubation effect due to slow drying. After four days in the drying-oven the soil is stored in waxed cardboard cartons [35] each containing about 750 g dry soil. These cartons are not airtight and the moisture content of the soil ultimately reaches equilibrium with the atmosphere of the store. Loam soils, after storage in this way, lose about 2% by weight when dried at 105°C. Such dry soil may be ground in an end-runner mill and any macro-organic matter finely divided and intimately incorporated; an essential requirement for analyses, such as that for organic carbon, which require an accurate sub-sample of 10 g or less.

Cold-storage of soils at -15°C. Soil samples fresh from the field are cold-stored in polythene bags. It is often convenient to pass the soil at field moisture content, through a 0.5-in. square-mesh sieve before storage. Sub-samples, as required for analysis (about 500 g), may then be divided from the stored sample without thawing the frozen soil. Cold-storage has

advantages over drying, particularly for analyses based on soil incubation techniques: the activity of micro-organisms is quickly reduced to a low level irrespective of the moisture content of the soil when sampled, the turf and many seeds are killed and the granulating action of freezing and thawing helps to standardize the physical condition of the soil. For these last two reasons it may even be preferable to cold-store soil for a short time rather than use soil fresh from the field (see also Gasser, 1958). The following data suggest that cold-storage for periods of up to two years does not appreciably reduce total microbial respiration during a subsequent period of incubation.

Soils stored at -15°C from Nov. 1956 to Nov. 1958	$\text{CO}_2\text{-C}$ output ppm. dry soil (20 days incubation at 30°C)
With addition of a fresh soil/water inoculum	452 ± 5.9
No inoculum	438 ± 5.9

Some slight micro-organic activity occurs during cold storage, including the period of freezing and thawing. In the following results from a series of soils sampled from swards in the year of sowing to ley, it is seen that ammonification was appreciable, but that nitrification was slight, over a 4-month period of cold-storage.

	ppm. of oven-dry soil	
	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$
Analysed direct from field plots	1.5	0.6
Analysed after 4 months cold storage	6.6	1.8

Methods of analysis

Analysis of soil for total organic carbon. The most convenient methods for estimating soil organic matter are those based on the reducing capacity of soil. This varies, however, not only with the quantity and quality of the organic constituents (Wesemael & Lehr, 1956) but also with inorganic constituents subject to oxidation or reduction. Carbonates do not interfere and such methods are of particular value for soils containing calcium carbonate.

Measurement of the degree of reduction of various oxidants after reaction in solution has been suggested from time to time. Normal $\text{K}_2\text{Cr}_2\text{O}_7$ was used in the method of Walkley & Black (1934). This reaction gave only about 70–80% recovery of soil carbon as estimated by dry combustion, and it may not satisfactorily digest fragments of root material. Schollenberger (1945) and Hester (1948) reduced bias due to incomplete oxidation of organic matter by increasing the concentration of the digestion mixture. The latter's method, which uses a 4N solution of sodium dichromate, was adopted for the routine analysis of soils from field plots.

Two hundred grams of soil dried at 30°C and passing a 0.5-in.-mesh sieve are ground in an end-runner mill to pass a 0.5-mm mesh. Roots and organic fragments are cut into small pieces before grinding.

Sufficient soil to contain about 0.04 g of carbon is weighed into a dry 8×1 in. Pyrex test tube. The soil is shaken with 5 ml of standardized

4*N* solution of sodium dichromate and allowed to stand until fully wetted. With each test tube standing in a spherical flask, to reduce variation in rate of cooling, 12.5 ml of Analar concentrated sulphuric acid are added over a period of about one minute. The flask is then gently shaken and put to one side. After 30 minutes the contents of the tube are rinsed into a 250 ml Erlenmeyer flask and made up with distilled water to a volume of about 100 ml. Residual dichromate is titrated against 0.5*N* ferrous sulphate using 1 : 10 phenanthroline ferrous complex indicator. Two analyses are made on each sample on different days. The standard deviation of blocks of analyses is calculated as $\frac{\text{mean range}}{1.13}$ (Snedecor, 1946). This estimate of

laboratory error is normally less than 2% of the mean carbon content of the soil: for pairs differing by more than twice this standard deviation a third analysis is made and the median value accepted.

To check the validity of the results obtained by dichromate reduction (method B in Table 2) analyses are made periodically on roots, macro-organic matter and soil, using a modification (Bremner, 1955) of a wet oxidation apparatus described by Clarke & Ogg (1942), in which carbon dioxide is measured gravimetrically (method A in Table 2).

It will be seen that the dichromate reduction method (B) provides an adequate estimate of the carbon present in grass roots and other forms of carbon accumulating in soil under leys. The lowest recovery was from soil under continuous annual tillage.

Table 2.

Carbon content of diverse soils and grass roots as determined by (A) wet oxidation and gravimetric measurement of CO₂ evolution and (B) dichromate reduction.

Source	Nature of organic matter	% carbon		$\frac{B}{A}\%$
		A	B	
Old pasture soil	Rich in decomposed organic residues	5.33	5.02	94
Arable soil	Organic residues resistant to oxidation	1.18	1.06	90
3rd-year-ley soil 0-2 cm horizon	Arable soil enriched with macro-organic matter and ley residues	2.63	2.58	98
Cocksfoot roots	Young roots from sand-cultured plants	46.4	47.8	103

Total soil nitrogen. Analysis for total soil nitrogen is made on 10 g of soil dried at 30°C and prepared as for total organic carbon analysis, using the Kjeldahl procedure for soils (Association of Official Agricultural Chemists, 1955) with a mercury catalyst.

The estimation of mineralizable nitrogen in soil ploughed from leys. Reviewing research over the past 40 years on the mineralization of organic nitrogen in soils, Harmsen & Van Schreven (1955) considered in some detail the incubation of soils under laboratory conditions. Despite the very artificial conditions of incubation they concluded that such techniques can effectively estimate the relative amounts of nitrogen that may be released from similar soils.

Cold-stored samples are broken to ensure rapid thawing and then crumbled and mixed by hand. Sub-samples of about 400 g are wetted on tension tables at zero tension for about one hour and then are allowed to equilibrate overnight at a tension of 75 cm of water. 300 g of soil at 75-cm moisture tension are introduced into 750-ml wide-mouthed Erlenmeyer flasks and at the same time a 50-g sample is dried at 105°C to obtain the moisture content. A 50-ml test tube containing 25 ml of 4*N* KOH is placed in each flask which is then stoppered with the T-junction leg above the level of the KOH (Fig. 9). After an hour in the incubator to allow the air to expand, the T-junction is inserted about 1 cm below the level of the KOH and the flask connected in series with an oxygen supply from a cylinder. Carbon dioxide evolved from the soil during incubation is efficiently absorbed by the KOH solution. Dependent upon the amount of oxygen consumed by the soil, a pressure deficit occurs

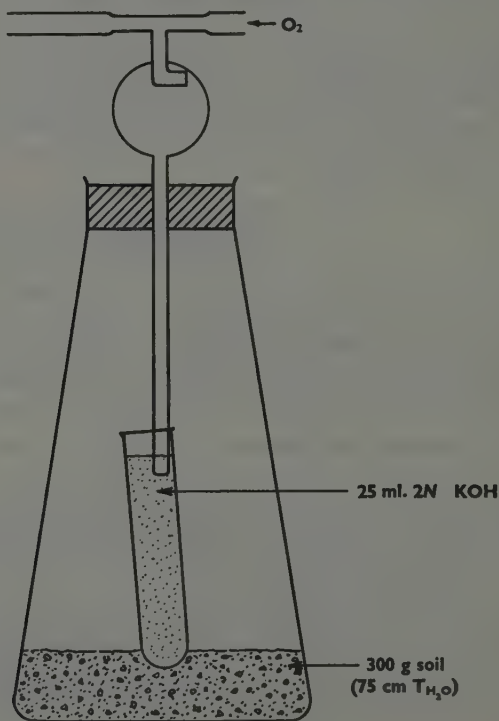


FIG. 9. Incubation unit.

within the incubation flask causing oxygen, supplied via the T-junction, to bubble through the KOH and maintain the partial pressure of this gas at its initial level. Incubation is continued for 20 days at 30°C, the system requiring no attention. The soil within the flask is then mixed and two 100-g samples are shaken for one hour with 200 ml *N* KCl solution acidified with HCl to about pH 1. Ammonia-nitrogen and nitrate-nitrogen are determined by hot distillation as described by Piper, 1950, pp. 208-11.

The results obtained by hot distillation were compared with those using the micro-diffusion method of Bremner & Shaw (1955). Both methods gave similar results. At the highest levels of mineral nitrogen (80-90 ppm.) recorded in soils from leys the estimate by hot distillation was about 3 ppm. greater than that by micro-diffusion.

To determine the amount of carbon dioxide evolved, the contents of the KOH tube are transferred to a beaker and brought to a total volume of about 100 ml with cold CO₂-free water. Titration with *N* H₂SO₄, using 1 ml of 0.33% phenolphthalein, to an end-point matching a standard buffered at pH 8.4 is followed by the addition of 1 ml bromophenol blue and further titration to pH 3.9. The quantity of acid used in the titration from pH 8.4 to pH 3.9 is an estimate of the amount of carbon dioxide evolved during the period of incubation.

The contents of the flask are sealed from the air and the moisture content of the soil has been found to remain fairly constant throughout the period of incubation. The danger of contamination is also minimized.

Variability between sub-samples of the same soil incubated at different times would seem to be related either to the level of mineralizable nitrogen present in the soil or to the previous agricultural management of the land:

Soil sampled from:	ppm. mineralized nitrogen	
	Mean	S.D. per single incubation
3-year ley, grazed	50.6	±8.3
3-year ley, cut	32.2	±5.0
Arable 4-course rotation	28.0	±3.0

The rate of mineralization of nitrogen and the production of carbon dioxide was found to be almost constant during the incubation of soils from leys for periods of up to three weeks.

Water-stable aggregation. A method of aggregate analysis by wet sieving is employed as described by Clement & Williams (1958).

CHAPTER 15

THE MEASUREMENT OF YIELDS OF CEREALS AND KALE AFTER LEYS

The growing, harvesting and weighing of cereals and marrow-stemmed kale on large numbers of small plots have led to the development of the methods described below.

The cereal plots are harvested with a self-propelled combine-harvester modified so as to deliver as accurately as possible the small amount of grain obtained from a plot. The straw, cavings and chaff are collected in a canvas sheet as they come off the straw-shakers of the harvester. Using the harvester, together with a tractor fitted with an hydraulically operated manure-loader to help weigh the straw, it is possible to harvest 80 plots, each 4×15 yd, in about 5 hr.

Marrow-stemmed kale is grown as a row crop and the crop is harvested when fully grown, using an adapted motor scythe. The kale is weighed in the same manner as the cereal straw. A sample area four rows wide ($8 \text{ ft} \times 15 \text{ yd}$) may be cut and weighed from each of 36 plots in 6 hr. Kale yields have also been measured by cutting a number of small samples by hand.

Harvesting cereals

General method

When crops follow a ley there is often a great deal of within-plot variation as a result of the uneven effects of the grazing animal. In order to get the true value of the effect of the ley treatment on subsequent crop yield it is desirable to harvest the whole plot. A second consideration is that when measuring plot yields it is necessary to discard the plot edges because of the propinquity effect of other treatments. It is especially important to do this in a rotation experiment where, during the course of the experiment, the soil is moved by ploughing after treatments have been applied. Our practice has been to discard a small area on the edges of the cereal plots and to harvest the remainder. The use of a modified combine-harvester satisfies the need for speed when harvesting a large number of plots.

Treatment and experimental design largely decide the size of plot and the lay-out; but lay-out and plot size considerably affect the time spent in preparation for harvesting and in harvesting (this is important in broken weather when trying to harvest a whole replicate in one spell), and this should be considered when designing the experiment.

Three methods of managing cereal plots are used. On plots 10×16 yd or 5×16 yd, most plot edges coincide with the former boundary of a ley treatment and 1.5 ft is discarded from the edges of such plots. A motor scythe [2], having a 3-ft cut, is taken down the intersecting lines between plots shortly before harvest and the cut material is cleared away by hand.

This preliminary clearing operation takes almost as much time and labour as the harvest itself. The edges of plots bordering wide races or bordering the outside of the experimental area are cut with the combine-harvester, thus avoiding hand clearing. Accurate trimming with the harvester is facilitated by fitting a single vertical sighting-bar in front of the driver. The sight is fitted to the guard-rail in front of the steering wheel and placed 1.5 ft in from the edge of the cut on that side. The driver keeps the sight in line with two distant sighting poles. For harvesting plots of this size, a combine-harvester, with a width of cut of 8.25 ft, must take two or more cuts along the length of the plot. See Table 3, Experiments H.8, H.9 H.9§, H.9*, H.16, H.16† for results.

The other two methods of management concern plots the width of the harvester or narrower. These methods are more convenient for the rapid harvesting of grain and straw and are used either when the ley treatments do not include the separate grazing of the plots (and the plots can be smaller for this reason) or when, after ploughing, large plots are split for different treatments to the cereal. Because the plots are narrow, they can be cut with one passage of the harvester and can be sited so that the distance the harvester has to travel over the whole experimental area is reduced to a minimum. Also the time spent in cutting and clearing races can be reduced. Because the performance of different coulter of a seed drill varies, it is best on long plots either to drill lengthwise, and arrange to have the same representation of coulters rows in each sampled area, or to drill across the plots, so that the larger number of shorter rows reduces the effects of coulters variation.

Lengthwise drilling is suitable when different treatments are applied by the action of the seed-drill, such as different rates of sowing, different fertilizers put on by a combined seed/fertilizer drill, or different times of sowing. In this method the plots are sown separately, by a drill approximately 3 yd wide (15 coulter, 7 in. apart) drawn along the length of each plot. Shortly before harvest, discard areas along the short sides of the plot are cut by the motor scythe, thus separating the plots at right angles to the future line of travel of the harvester. The discards from the long sides of the plot are quickly obtained by cutting with a sickle, or even by treading down the two edge rows, one on either side of each passage of the drill. The method gives a smaller discard than that of the larger plots, there being one 'guard' row on each side between the harvested area and the adjacent treatments; however there is usually less need for a large discard and there is flexibility—more rows can be discarded, or, in certain circumstances, the gap between each passage of the drill can be increased. See Table 3, Experiments H.67 and H.180, for results.

The third method is suitable when treatments are not to be applied by the seed drill and drilling can then be done uniformly over the whole area, across rather than along the plots. Our treatments in this case have been different levels of a seed-bed fertilizer, put on by hand. The only races necessary are those separating the plots across the line of travel of the harvester. These are cut with the motor scythe. A discard of any size decided upon when the plots are laid out can be left on either side of the harvested area. At a minimum the plot need be only one foot wider than

the width of the harvester. The harvester is steered on a straight course through the plot leaving a discard standing on both sides, the driver using the sighting bar. See Table 3, Experiments H.8* and H.26, for results.

Modifications to the combine-harvester. A self-propelled combine-harvester [20] with an 8.25-ft cut is used. Modifications allow the collection of the small amounts of grain, between 15 and 80 lb, from individual plots: and they largely eliminate the contamination of grain from one plot by that from another.

Normally the grain passing the sieves of the machine falls into the channel of a grain auger. The auger, which lies horizontally across the harvester, pushes the grain to an elevator which conveys it to hoppers on the bagging-platform. Pockets of grain tend to collect in the housings of the auger and elevator and it is impossible to clear these between plots. Modification allows the grain to pass from the auger channel into a box fitted beneath, thus avoiding the use of the elevator. On completing a plot the grain is discharged from the fitted box in less than one minute. The conversion from plot working to normal farm working and back again takes only a few minutes. The modifications necessary are as follows:

1. A panel 3.75×21 in. is cut from the rear side of the channel of the grain auger. In order that the harvester can function normally when in farm use, a metal panel slightly larger than the one removed is hinged along the ridge of the channel so that it can be closed and fastened over the hole left by the removal of the panel. When the machine is in use on plots the fitted panel is unfastened and remains open allowing grain to fall past the auger through the hole.
2. A metal box completely open at the top is fitted to the framework of the harvester in a position to catch grain falling through the hole in the auger channel (Plate 13). The box, measuring 23 in. (across the harvester) $\times$ 20 in. $\times$ 10 in. in height is of sufficient capacity to contain 80 lb of grain, an amount not likely to be exceeded from a plot of 60 sq. yd. The box is anchored at its foremost end by two flat-iron hooks attached to the box, which fit over a U-section iron cross-member of the harvester framework. The after-end is secured by two small brackets which can be seen in Plate 13. In order to expedite the discharge of grain after cutting a plot, the box is fitted with a sliding bottom (Plate 13), which allows it to be quickly emptied. Because of the weight of grain, this bottom needs support from all four sides when the box is closed. The box may be left on the harvester when it is in normal farm use; but, if necessary, it can be removed in 10 minutes. Its ground clearance is 1 ft.
3. In order to receive the grain on its discharge through the bottom of the fitted box, a metal tray was made, 8 in. deep and 31 in. square with a funnel extension at one end (Plates 13 and 14). With the harvester stationary, the tray, resting on the ground, is pushed under the fitted box. The tray is designed to allow the grain to be shot quickly into a sack hooked beneath the funnel (Plate 14).

Arrangements for the collection and weighing of straw. It is desired to collect the straw including the cavings and chaff and to weigh the produce

from each plot. Weights up to 160 lb are dealt with quickly. This is the maximum amount likely to be obtained from an area of 60 sq. yd. The following arrangements are made:

a. Four chains, terminating in snap links, are fixed to the framework at the rear of the harvester. A canvas sheet is hitched to the chains to collect the straw, cavings and chaff coming off the straw-shakers. The positioning of the chains can be seen in Plates 15 and 16.

b. The canvas sheet measures 11×8.75 ft. Steel rings are sewn at each corner and two additional rings are sewn on one short side, each 1.5 ft from the middle of that side. This side is fastened by the chains to the rear of the harvester (Plates 15 and 16). The lower chains are attached by the snap links to the two middle rings and the upper chains to the outside rings. If two such sheets are available the straw from one plot can be weighed and sub-sampled while the harvester cuts the next.

c. A spring balance is suspended from a tractor-mounted hydraulically operated manure-loader. Plate 16 shows the arrangement. A tubular bar is fitted over the central prong of the loader. The weight is distributed over the other prongs by a second bar welded on about half way down the tube and at right angles to it. Two hooks are welded to the end of the tube, one to suspend the spring-balance and the second, immediately adjacent, is used when transporting the straw and avoids jarring the balance. This second hook is an innovation not shown on the Plate. The sheet, loaded with straw, is hoisted on the balance and weighed using the controls of the tractor in the normal way.

Operation

From 6 to 8 men (including drivers) are needed if the grain is to be left sacked, the straw weighed and put on the headland and a sub-sample of straw drawn and sacked. If the grain alone is collected, the harvester driver and two men are enough.

Before starting on the plots the harvester is operated for a short distance on the headlands of the crop. Then the hinged panel in the auger channel is opened and the machinery of the harvester is run for 45 sec to clear the channel. The machine is then stopped, the box is fitted and the sliding bottom pushed home. Finally the grain hoppers at the bagging platform are cleaned and new sacks fitted. These are examined at the end of each plot as a small amount of grain (less than 0.25 lb) may be thrown into the elevator by the grain auger: it is recovered in the sacks and returned to the sample. This could be avoided by blanking off the bottom of the elevator, but the amounts of grain involved are too small to make it worthwhile in our experiments.

The harvester is now placed in position to cut the first plot and the canvas sheet is clipped into place. As the plots are cut the sheet is trailed out and held to catch the straw (Plate 15).

After finishing a plot the threshing drum and the shakers and sieves are kept running. The sheet filled with straw is uncoupled and hoisted on the hydraulic lift of the tractor. Another sheet is then fitted to the harvester. During this time the grain tray is slid under the fitted box and

the sliding bottom of the box is withdrawn, thus shooting the grain into the tray (Plate 13). Nearly all the grain comes through the machine within 20 sec of the end of the cut; however the drum is kept running and a timed interval of 45 sec is allowed to elapse before the sliding bottom is replaced in preparation for the next plot. The tray is then pulled out from beneath the harvester which may then proceed. The discharge of grain and straw takes about one minute. During the cutting of the next plot the grain from the previous one is sacked and the straw is weighed, put aside, and a sub-sample drawn. The moisture content of this sample is measured and subsequently related to the field weight. The grain is stored to be weighed later.

In this way 80 plots 4×15 yd in area are harvested in 5.25 hr. If the grain alone is collected, 4 hr are taken. The plots require two cutting journeys of the harvester. On narrower plots, i.e., those which are the width of the harvester $\times 9$ yd long, 80 are harvested in 3 hr, if the grain alone is collected.

Error control

Using the methods outlined, the plot errors and coefficients of variability were obtained as shown in Table 3.

Table 3.
Variability of estimates of cereal yields
Yield of grain (14% moisture)

Experi- ment	No. of years	Mean yield cwt/acre	Total no. of plots	Plot error cwt/acre	Coefficient of variability %	Size of harvested area, in yd
GRAIN						
H.8	3	30.4	360	$\pm 2.78\uparrow$	9.1	15×4
H.8*	1	37.1	300	$\pm 3.02\uparrow$	8.2	9×2.75 (harvester width)
H.9	3	36.7	96	$\pm 1.49\uparrow$	4.0	15×9
H.9§	2	40.7	64	$\pm 1.66\uparrow$	4.1	15×4
H.9*	2	33.9	64	$\pm 1.18\uparrow$	3.5	15×4
H.16	3	32.1	60	$\pm 2.81\uparrow$	8.8	15×4
H.16†	3	26.1	60	$\pm 1.52\uparrow$	5.8	15×4
H.26	1	28.4	48	± 1.62	5.7	7.5×2.75 (harvester width)
H.67	1	42.0	24	± 2.48	5.9	9×2.5 (13-coulter width)
H.180	1	33.5	32	± 1.43	4.3	9×2.5 (13-coulter width)
STRAW						
H.9	3	32.9	96	$\pm 2.32\uparrow$	7.0	15×9
H.16	3	37.4	60	$\pm 3.19\uparrow$	8.5	15×4
H.180	1	39.3	32	± 3.41	8.7	9×2.5 (13-coulter width)

*† All crops were winter wheat sown directly after ley with the exception of *, spring barley after kale after wheat after ley and †, winter wheat after wheat after ley.

† These experiments contain split-plot treatments. The split-plot errors are given.

§ The H9 and H9§ results were obtained on different plots in different years.

It should be noted that certain experiments contained split-plot treatments and the resultant errors are likely to be reduced for this reason. The yield of each plot was calculated after the determination of the loss of weight on drying at 100°C of sub-samples taken from the sacks of grain on a convenient day after harvest and from the straw in the open at the time of harvesting. The grain is easy to sub-sample accurately, but it is difficult to obtain a representative sub-sample of straw, including the cavings and chaff. This enlarges the plot error for straw.

The effect on variability of a single factor such as plot size is complicated because the effects of years and of experimental treatments, different for each experiment, interact with other factors affecting total variation. It will, however, be seen that quite reasonable error control was obtained from plots 2.5 yd (13 coulter rows) $\times$ 9 yd and from plots 2.75 yd (harvester width) $\times$ 7.5 yd.

Harvesting kale

General method

Marrow-stemmed kale is normally our second crop after ley. The kale is sown in rows 2 feet apart and harvested in December when it is fully grown. Plots of two sizes, 10 $\times$ 16 yd and 5 $\times$ 16 yd are used. Because of the difficulty of handling kale it is not practicable to cut and weigh the whole plot. Samples are therefore cut from the standing crop and these are weighed in the same way as the cereal straw, *q.v.*

Two methods of cutting have been used. In the first of these a number of individual samples, each 3-ft wide across 4 drill rows (3 $\times$ 8 ft), are cut by hand from random sampling positions in each plot. This size of sample is convenient because a 4-coulter drill is used at sowing and care can therefore be taken to have the same representation of coulter rows in each sampled area. It also allows for a propinquity effect on the yields of contiguous rows. Usually 15 samples are taken from a plot 10 $\times$ 16 yd. It is likely that the number of samples could be reduced with little increase in error. For two years samples were taken at an earlier stage of growth, in September, when 7 samples only were taken. Because of the difference in the stage of growth of the crop the data are not strictly comparable but the smaller number of samples led to little increase in the coefficient of variability (Table 4).

Hand cutting is laborious, as is collection from random sampling positions before weighing. However, the method has the advantage that the sample has a wide distribution. The plot edges can also be avoided in sampling, so that there is no need to cut races.

In the second method the kale is cut using a motor scythe [2] with an attachment. Because the scythe works best along the rows, the plots are drilled lengthwise; on a plot 10 $\times$ 16 yd, 4 contiguous rows from one passage of the seed drill are cut the length of the plot, to estimate yield. Thus an area 15 yd $\times$ 4 rows (40 sq. yd) is harvested. There is not the same wide distribution of sampled area within a plot as with the first method although the error control has been satisfactory (Table 4). Samples from a single large area are quickly collected for weighing. Before harvest

it is necessary to cut and clear discard strips, or races, along the short sides of the plots, in the same way as for the cereal crop. This is done with the motor scythe. The machine will cut a 3-ft wide race *across* the rows but does not then form a swath, and this makes clearing more difficult.

The motor-scythe attachment. The attachment that serves to deflect the cut kale past the wheels of the scythe is shown in Plate 22. A steel bar 2 in. $\times$ 0.25 in. in cross-section forms the central part of a framework fitted to carry two 0.75-in. L-section iron arms which deflect the kale. The framework, which is strengthened by a number of welded round-sectioned rods, is fixed to the motor scythe by 4 steel straps. Two of these are bolted below the petrol tank and two, which are vertical, immediately behind the reciprocating knife. Some adjustment is provided by alternative bolting-up positions on the vertical straps. The L-section iron arms project from the axle stubs on each side of the scythe to converge 3 ft 8 in. in front of the cutting knife and at axle height. A welded rod gives support from the framework at this point. A number of bolting-up positions is provided for the two 5/16-in. bolts securing each L-section iron arm to the framework, at the axle end. Thus the arms can either be off-set (as in Plate 22) for use with the side-cutting knife (this allows continuous working along two rows) or the arms can be positioned centrally. The central position is only useful when cutting across rows (when cutting the races for instance): with the knife also central a 3-ft wide continuous cut can be made through the crop, throwing the cut kale to each side. The attachment could be more rigid but is otherwise satisfactory. The long arms are a hindrance where turning round in a standing crop but the arms must extend well in front of the knife. Constructional details have been described by Hopper *et al.*, (1960).

Arrangement for weighing the kale. The same canvas sheets are used as for holding the cereal straw. Each sheet when filled is hoisted and weighed using the tractor-mounted manure-loader. The area harvested from a plot 10 $\times$ 16 yd by either sampling method is 40 sq. yd and the field weight of crop approximately 450 lb. This amount can be managed easily and

Table 4.

Results obtained from harvesting marrow-stemmed kale on plots 10 $\times$ 16 yd

Experi- ment	No. of years	Mean D.M. yield lb/acre	Total no. of plots	Plot error lb/acre	Coefficient of variability %	Size of harvested area, in ft
H.9	3	6880	96	$\pm 351\ddagger$	5.1	15 samples each 3 $\times$ 8, by hand
H.9*	2	5160	64	$\pm 297\ddagger$	5.8	7 samples each 3 $\times$ 8, by hand
H.16	2	7690	40	± 488	6.3	8 $\times$ 45, by motor scythe
H.8	2	7870	120	$\pm 434\ddagger$	5.5	8 $\times$ 45, by motor scythe

* Data are from the H.9 plots but sampled at an intermediate stage of growth.

† These experiments contain two split-plot treatments. Split-plot errors are given.

quickly in two weighings. After weighing, sub-samples of about 40 lb are drawn and chopped in a chaff-cutter. The chopping is done to give accuracy to the further sub-sampling which is necessary. The yields are calculated, after measuring the loss of weight on drying at 100°C, of two 1000-g sub-samples from the chopped material from each plot.

Operation

A tractor driver and 4 or 5 men are needed whichever method is used. They will weigh the kale, put it on the headland and leave a sub-sample drawn and sacked.

With the hand-cutting method the kale is cut with a root hook. The separate samples are picked up by hand and brought to the edge of the plot where they are piled into the canvas sheet for weighing. To avoid the mistake of picking up a sample from the wrong plot alternate plots are cut and weighed first and the remainder are dealt with later. Cutting 15 samples in each plot, 6 men, including the tractor driver, cut and weigh the kale from 20 plots in 6 hr.

In the second method one operator with a motor scythe can cut two rows of kale at a walking pace even when the soil is wet. The scythe, cutting along the rows, leaves a neat swath that is convenient for collection. Cutting an area of 40 sq. yd in each plot, 5 men, including the tractor driver and motor-scythe operator, sample 36 plots in 6 hr.

The coefficients of variability obtained with the methods are given in Table 4: they are reasonably low.

CHAPTER 16

AN APPARATUS FOR SMALL-PLOT IRRIGATION

Commercial equipment for the spray irrigation of farm fields is not suited to experimental work on a small scale, by reason of the uneven distribution of water within plots, the inconvenience of accommodating a large area of partly irrigated land between irrigated and non-irrigated plots (because of the fringe region of the sprays), and the restriction of irrigation to periods of calm weather.

For critical work with small plots, it is necessary to use special equipment having the following attributes: it must apply water uniformly over the whole area of a plot; the quantity of water applied should be accurately and easily measured; as little water as possible should fall outside the confines of the plot; the equipment should be easy to move from one plot to another; the spray should be insensitive to wind; the design should not present too severe a challenge to the workshop facilities available for its construction and maintenance.

The apparatus to be described has been in use at the Grassland Research Institute for some years and fulfils the above requirements adequately. Its basic design is due to Myers (1952), but the details of construction have been modified here in the hope of improving uniformity of water application, increasing portability, and making it suitable for available facilities.

General description

The apparatus is shown in Plate 17. A bridge girder, made of L-section aluminium spans the plot lengthways. The ends of the girder are supported by L-section aluminium legs, which are provided with carrying handles, and feet which can be adjusted to set the apparatus level. A wheeled carriage travels slowly back and forth along the bridge girder and carries a spray pipe which spans the plot crossways.

A portable petrol-electric generating set [1] supplies power to a geared electric motor which drives a winding drum, which in turn pulls the carriage along by a wire rope which passes from the drum to the carriage, whence a second rope passes round a pulley at the far end of the bridge girder, and back to the drum. When the carriage reaches the end of its travel, it operates a double pole changeover switch which reverses the current through the armature of the motor and so sends the carriage back.

To provide a supply of water at constant pressure, a small header tank is mounted at one end of the bridge girder. Water from the mains enters the header tank through a float valve, and flows from the tank through a water meter towards the centre of the bridge girder where it enters a swinging arm of brass tubing. The swinging arm is supported by coil springs in tension connected between a crosspiece on the arm and anchoring points on the bridge girder. The free end of the swinging arm is connected by hose to the down-pipe by which the spray pipe is fixed

to the carriage. The down-pipe contains a wire-gauze filter to protect the spray holes from dirt in the water. The spray pipe consists of a thin-walled brass tube, with a large number of small holes drilled in a single row on its under side, so that the water leaves the pipe in a large number of vertical streams. A few inches below the pipe, the water breaks up into droplets.

Design considerations

Plot size

The size of plot must first be decided on, since, once the apparatus is built, it is not readily adaptable to varying plot sizes. We use plots 18×12 ft and consider this to be about the most suitable size for this apparatus. It could be increased slightly. Myers used plots of 24×16 ft, but the extra weight and reduced rigidity of the structure needed for this size of plot would make handling considerably more difficult. The weight of our apparatus is 1.5 cwt, which is about as much as two men can carry without strain. If the apparatus is made smaller there will not be a proportionate saving in cost and two men will still be needed to move it. If smaller plots are desired, it is worth considering whether a split-plot lay-out is appropriate to the proposed experiments, with, for example, several fertilizer treatments grouped in one irrigated plot 18×12 ft, since in this way the fullest use can be made of the apparatus.

Watering rate

The rate of water application should be made as fast as is permissible to reduce to the minimum the time spent on irrigation, or the number of machines needed to cope with the experimental programme. We use a rate of 1 in. of water in 35 minutes, which is satisfactory for our loam of about 15% clay content with a complete cover of established herbage, but slower watering may be required when heavier soil or bare soil is to be irrigated.

The rate of application of water is given by the formula $R = d^2 h / 180 \cdot t \cdot s$, where R is rate of watering in inches per hour, d is nominal diameter of drill used for spray holes in thousandths of an inch, h is height of header tank above spray pipe in feet, t is length of travel of spray pipe in feet, and s is spacing of holes along spray pipe in inches.

For example, we use $d = 28$ (No. 70 drill = 0.028 in.), $h = 7$ ft, $t = 18$ ft, $s = 1$ in. This gives $R = 1.7$ in. per hour, approximately.

As a rough indication, apparatus working at this rate will serve 20–30 irrigated plots in conditions where potential transpiration rarely exceeds 1 in. per week, and the average amount of water applied to one plot at one time is 1 in.

The best way to increase the watering rate would be to use larger spray holes, which are easier to drill and less likely to get blocked than small ones. In the more likely case of a reduced rate being required, the best procedure would be to increase the hole spacing to 2 or 3 in., and to decrease the hole size only if still slower watering is needed.

There is little scope for varying the height of the header tank, as this has to be a little higher than the maximum height of the swinging arm,

in order to avoid the risk of air locks in the latter; it would be structurally unsound if made much higher.

If more than one rate of watering is required, it would be best to make a separate spray pipe for each rate, and fit a union in the pipe just below the filter, so that it can readily be changed.

Water consumption

With a spray-pipe length of 12 ft, a travel of 18 ft, and a watering rate of 1.7 in. per hour, water consumption is about 190 gallons per hour. The uniformity of watering will suffer if the water-supply pressure is too low or the pressure drop in the supply pipe is too high for the designed flow rate to be achieved. This affects the size of water pipe to be used in relation to its length and to the supply pressure. With a mains pressure of around 20 lb per sq. in. we bring the water to the field in 2-in. pipes, and connect to the irrigation apparatus with 200 ft of 1-in.-bore polyvinyl chloride hose. Formulae or tables of pressure drop in pipes can be found in most handbooks of mechanical engineering.

Header tank

In view of its location and weight, the header tank should be kept as small as possible. The minimum size of the float is controlled by the size of the needle valve orifice and the maximum water pressure when the needle valve is shut. The size of the valve orifice is determined by the required flow and the minimum water pressure when the valve is open. The 3/16th-in. orifice shown in Fig. 10 is designed to pass 200 gal per hr at 10 lb per sq. in., and the 4×4 in. float, made of expanded ebonite [21], is designed to close the valve against a static head of 30 lb per sq. in. It will be seen that if the water supply pressure is very variable, or the pressure drop in the pipes is very large, the header tank will need to be larger.

Speed of travel

The winding drum rotates at 15 r.p.m. to propel the carriage from one end of the bridge girder to the other in one minute, but this speed is not critical. Constant speed is important for uniform watering, and is obtained by using a shunt-wound motor with an ample reserve of power, and a generating set with an electric governor intended to give constant output voltage.

Constructional details

Bridge girder and legs

These are rivetted assemblies made of 2 in. × 2 in. × $\frac{3}{16}$ in. and 1 in. × 1 in. × $\frac{1}{8}$ in. L-section aluminium alloy. The bridge girder is 20 ft long for an 18-ft plot. Each pair of legs is bolted to the bridge girder, so that it can be removed for transport and storage. Tubular carrying handles are fitted to the legs. The ease of moving the apparatus depends to a large extent on the comfort of the handles.

Carriage

The carriage frame is made of 2-in. L-section aluminium alloy. Short lengths of aluminium are welded to the upper horizontal member to

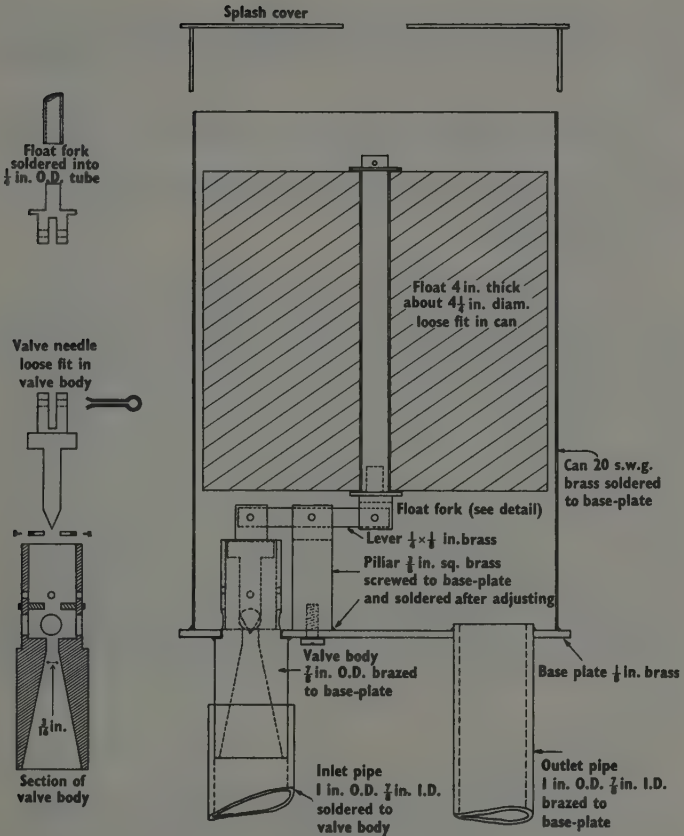


FIG. 10. Header tank assembly.

form box sections where the wheel spindles are fitted. The vertical member is rivetted to the upper horizontal member, but bolted to the lower one, so that the latter can be detached to enable the carriage to be removed from the bridge girder. The carriage is steadied by two small horizontal wheels, cut from 1.5-in. light alloy bar. All the wheels are fitted with nylon bushes, running on fixed stainless steel spindles without lubrication. The driving ropes are joined to the carriage by rigging screws, with which the rope tension is adjusted (Plate 18).

Carriage driving mechanism

Plate 19 shows how the electric motor is fixed to the bridge girder with wing nuts, and connected electrically by means of a four-way plug and socket, so that the motor may be taken indoors when the apparatus is not in use.

The mechanical efficiency of geared motors with low output speeds is poor; it is therefore desirable to achieve part of the speed reduction with a belt drive.

The winding drum, Plate 20, is made from a triple V-belt pulley, by removing the intermediate flanges. The wire rope is $\frac{3}{32}$ in. diameter, 7×7 strand stainless steel.

Reversing mechanism

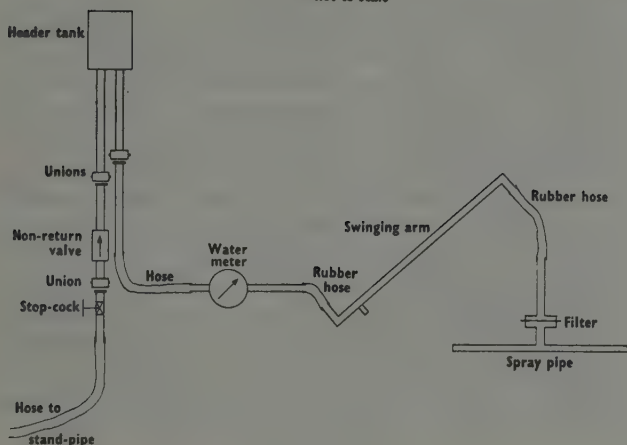
The electric circuit is shown in Fig. 11, and the mechanical arrangement of the reversing switch in Plate 20. Two changeover pattern mercury switches are held in spring clips on a T-shaped assembly of brass sheet which is mounted so that it can be tilted to bring either end of the mercury switches horizontal. A coil spring in tension between the top of the T-piece and the switch base-plate gives the T-piece two stable positions which are adjusted by horizontal screws working in nuts brazed to the base-plate. A thin brass rod, supported in several brackets, runs the length of the lower angle of the bridge girder. One end of the rod passes through a slot in the tail of the T, to which its endwise motion is transmitted by collars on the rod and coil springs (to cushion the shock). Rotation of the rod is prevented by fitting it, near one of the brackets, with a square collar which works against a short length of aluminium bolted to the bridge girder. The carriage is fitted with two small brackets, visible in Plate 18, on either side of the horizontal guide wheel. When the carriage reaches the end of its travel one of these brackets strikes a trigger fixed to, and projecting downwards from, the switch rod, thus operating the switch.

Water system

A schematic diagram of the water circuit is given in Fig. 11, and its physical form is shown in Plates 17 and 18.

It is usually necessary to disconnect the hose when the irrigator is moved, so a stopcock is permanently attached to it, and fitted with a union on its outlet side by which it may be detached. The other part of the union is fixed to a non-return valve which prevents the operator getting drenched when undoing the union. The vertical pipes to and from the header tank are fixed to the leg assembly and include unions about 5 ft above ground level by which the header tank assembly may be detached. The internal components of the header tank, as shown in Fig. 10, are assembled onto the base-plate; then the cylindrical can is soft-soldered to the base-plate, so that it is not too difficult to remove it if access to the interior is needed. The water flows from the header tank to the water meter which rests on, but is not fixed to, the crosspiece of the legs and is fixed in position by its outlet pipe. The latter is a horizontal brass tube with brazed-on lugs by which it is screwed to the lower member of the bridge girder.

Schematic diagram of plumbing
Not to scale



Wiring diagram

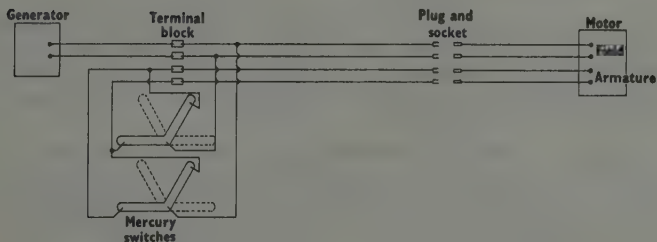


FIG. 11. Irrigation apparatus—pipework and wiring diagram.

Its inlet end is bent, to bring the water meter to a convenient position, and its outlet end, near the centre of the bridge girder, is bent through a right angle in the horizontal plane, so that the hose connection to the swinging arm forms a semicircle.

It is essential that both hose connections to the swinging arm are made of non-kinking hose. Double-braided rubber hose is satisfactory. The swinging arm is a brazed assembly of brass tube and rod. For 18 ft travel, the length from the pivot to the free end is 5 ft 2 in., the two ends

of the arm, to which the hoses are fitted, being at right angles to the arm itself (Plate 17).

The water filter, just above the centre of the spray pipe, is a cylindrical brass box, made in two halves clamped together with 8 screws. The filter gauze is held between cork gaskets between the two halves. The apertures in the gauze should be considerably smaller than the spray holes. To protect No. 70 or 72 drill holes, the coarsest gauze is 60 mesh per in. If there is much dirt in the water, it would be advisable to fit a second filter, of an easily cleaned type, upstream of the water meter.

The spray pipe should be thin-walled for ease of drilling, and large enough to give reasonable mechanical strength and a negligible pressure drop from the centre to the ends of the pipe. We use $1\frac{1}{4}$ in. O.D. $\times$ 22 S.W.G. hard-drawn brass tubing. The ends of the pipe are closed with screw plugs, made from electrical conduit fittings, so that the pipe may be flushed. Drilling the spray holes requires care, but it can be done with ordinary workshop equipment. We have successfully used No. 72 drills in a $\frac{1}{2}$ -in. capacity pillar drilling machine, and No. 70 drills are appreciably more robust, but if much smaller holes are required, a smaller high-precision drilling machine would be essential. After drilling, an attempt is made to clear the burrs from the inside of the pipe with a pull-through, and the holes are cleared again with a drill held in a pin chuck.

Adjustments

Levelling the apparatus. The apparatus must be level if water application is to be uniform, but it is normally sufficient to judge it by eye, sighting in turn along the bridge girder and spray pipe on some feature of the landscape, such as a distant fence.

Driving belt. The tension in the belt drive from the motor to the winding drum is set so that the belt does not slip in normal operation, but will slip, and thus prevent damage to the apparatus, if the carriage meets an abnormal resistance. A new belt usually stretches and needs shortening after being in use a short while.

Swinging arm. The hose from swinging arm to spray pipe is adjusted so that it is just long enough to have no tendency to lift the carriage off the bridge girder. The springs which hold the swinging arm are tightened so that when the arm swings over at the middle of the carriage travel, the free end of the arm stops about 1 ft above the bridge girder.

Wire rope. The tension in this is light. It is sufficient to tighten the rope so that its lower run does not drag on the bridge girder.

Length of travel. This is set to its designed value by moving the triggers on the rod that tilts the reversing switch. It must be remembered that changing the length of travel produces an inverse change in the rate of water application.

Spray pipe. When water is first turned on, some of the spray holes will be seen to be blocked. Most of these can be cleared by removing one of the end plugs, and tapping the underside of the pipe in several places with a

screwdriver handle, working from the centre to the open end, so that the dirt is dislodged and flushed out by the water. After similarly treating the other half of the pipe, any holes which are still blocked, or delivering a squirt sideways, are cleared by using a drill held in a pin chuck, and the flushing procedure repeated.

List of materials for one apparatus, for irrigating plots measuring 18×12 ft

1 electric motor 110V d.c., shunt wound.	17 lb. in. at 51 r.p.m.
1 1 in. rotary water meter.	
Aluminium alloy L-section	2 in. $\times$ 2 in. $\times$ $\frac{3}{16}$ in. 50 ft.
	1 in. $\times$ 1 in. $\times$ $\frac{1}{8}$ in. 100 ft.
Aluminium rivets	$\frac{1}{4}$ in. diam. $\times$ $\frac{1}{2}$ in. long 1 gross.
	$\frac{1}{4}$ in. diam. $\times$ $\frac{5}{8}$ in. long 1 gross.
Aluminium nuts & bolts	$\frac{1}{4}$ in. B.S.F. $\times$ 1 in. long 6 doz.
Brass tubing	1 $\frac{1}{4}$ in. O.D. $\times$ 22 S.W.G. 12 ft.
	1 in. O.D. $\times$ 16 S.W.G. 40 ft.
Brass rod	$\frac{1}{8}$ in. diam. 30 ft.
Stainless steel rod	$\frac{5}{8}$ in. diam. 5 ft.
Nylon rod	20 mm diam. 1 metre.
6 Plummer blocks $\frac{5}{8}$ in. bore.	
3 M section V-belt pulleys 4 in. diam.	
1 A " " " " 2 in. P.C.D.	
1 A " " " " 7 in. diam.	
1 A " triple pulley 6 in. diam. (for winding drum).	
$\frac{1}{2}$ in. link-type V-belt 4 ft.	
$\frac{3}{8}$ in. flexible wire rope 60 ft.	
2 changeover pattern mercury switches (plus spares).	
1 4-way plug and socket.	
4-way flex, 6 ft.	
3-way flex, say 15 ft.	
2 springs, 15 S.W.G. $\frac{5}{8}$ in. diam. 1 ft long, close wound (size not critical).	
Double-braided 1-in. bore rubber hose 10 ft.	
1-in. bore plastic hose, according to need, up to 100 yd.	
12 worm-drive hose clips, for above.	
4 1 in. hose-to-hose unions (plus plenty of spare washers).	
1 1 in. stop-cock.	
1 1 in. vertical check valve.	
Brass sheet 20 S.W.G. about 1 sq. ft.	
" " $\frac{1}{8}$ in. about 1 sq. ft.	
Drills for spray holes, say 1 dozen.	
Small quantities of brass rod, filter gauze, piano wire, screws, etc.	

PART V

EXTENSION TRIALS

Experimental work at research institutes often lacks the variations in environment that may occur in farming. The object of the trials in the Department of Extra-mural Studies is to test promising results obtained at the Grassland Research Institute under a wide range of environmental and practical farming conditions. It may also be necessary to carry out trials (e.g. on the control of a specific weed of permanent grassland) for which facilities are not available at a research institute. Finally, research projects (e.g. on local soil problems) may have to be carried out at the localities where the problems occur.

CHAPTER 17

PLANNING AND OPERATING EXTENSION TRIALS

The extension trials of the Institute are generally situated on commercial farms. This type of research demands considerable attention to detailed planning and organization, otherwise a large proportion of the centres may have to be discarded because of the application of incorrect managements, etc. In addition to the active co-operation of participating farmers, it is desirable to have local advisory or technical officers who are in constant close touch with the farmers and who can supervise the day-to-day managements. It is beneficial to discuss trials at an early stage in planning with co-operating technical or advisory officers and before drawing up the final details. These officers may indicate useful additions to, or modifications of, the experiments.

Planning and schedules of experiments

Two sets of plans are drawn up. The first is detailed and includes reasons for the experiment. This is circulated to the co-operating technical or advisory officers so that they can appreciate the project and the management problems involved. These officers make the initial selection of co-operating farmers and also discuss with them the salient features of the experiment. A second set of plans is available for the farmers and indicates very simply the treatments and various requirements of the experiment. Reminders of various events in the experiment are circulated frequently to the co-operating farmers and advisers; this not only avoids mistakes but also maintains active interest. Because of the importance of maintaining interest, interim reports of the experiments are prepared and circulated as rapidly as possible. Co-operation with local officers is an invaluable asset in the running of extension trials, but the Institute maintains

responsibility for the co-ordination of the trials and for the sampling and interpretation of the data. This ensures: (1) that the sites selected bear a correct relationship to the object of the experiment; (2) that the sampling and collection of data are uniform; (3) that all the centres are known to the person who interprets the results.

Selection of sites

The selection of sites within any set of environmental conditions is determined by three factors. Firstly, the local officer should have a direct interest in the experiment. Secondly, the farmer must be both co-operative and capable of performing any necessary managements. Experience has shown that generally farms of 100–500 acres are the most useful. Below this size the farms are usually too small to allow the flexibility in management which may be necessary to complete an experiment successfully. On larger farms the owner tends to delegate responsibility to one or more managers and personal interest is often absent, so that direct contact with the person managing the experiment is more difficult. Finally, the selection of the site is obviously of prime importance and must bear a close relationship to the requirements of the experiment. Care is taken to check not only the past history of any potential sites, but also the possible future plans of the farmer (e.g. the outdoor feeding of silage could affect the movement of cattle over a wide area).

Obviously experiments should be sited to avoid gateways, paths, etc., and the lay-out will have to take into account the gateways and the availability of drinking water for stock. Where the entrances and water points are limited, corridors may have to be provided for access to individual paddocks. This is normally not a difficult task if electric fencing is available. When particular managements are required on certain plots it is better that the experiment should occupy the whole of a field, or should be permanently fenced off, otherwise the experimental area may inadvertently receive treatments intended for the remainder of the field.

Accessibility to the site in all weathers is of major importance. Although accessibility is important it is usually desirable to ensure that the trial is not carried out in the home-farm paddock; during the summer this normally carries too much traffic and sick or convalescent animals may be added "just for a few days". The fencing around a site must be adequate, not only to cope with any experimental animals, but also to keep other livestock out.

Marking of plots

A useful semi-permanent method of marking plots in grass swards is to use wooden pegs (1.5 in. square $\times$ 12 in. long) which are knocked in almost to ground level to avoid machinery. Any suitable herbicide which kills all vegetation is then sprinkled round the pegs which can readily be found and identified. For experiments requiring more permanent markers, e.g. where experimental plots may be ploughed, 6-ft stakes made from L-section iron are driven well into the ground at the field boundary to form a known datum line. The tops of these stakes are painted with fluorescent paint for easy identification.

CHAPTER 18

EXPERIMENTAL DESIGN

Experiments to compare contrasting treatments over a wide range of environmental or farming conditions are conducted in order to determine whether the application of particular managements on a farm scale gives the same results as those obtained at the Institute. For this reason it is often requested that the treatments (e.g. fertilizer or grazing managements) should be applied by the farmer, which necessitates a small number of simple treatments. It is generally undesirable to request the farmer to apply more than two or three treatments at once, and, in order to fit in with normal farm practices, the individual management areas should be of reasonable size (e.g. at least 2 acres). It is, however, possible to superimpose simple managements so that a wide range of treatments is obtained. Fig. 12 shows how the application of fertilizer at two dates to different areas and also the cross-application of two grazing treatments give a large number of individual treatments whilst involving the farmer in the minimum of trouble. This lay-out has the disadvantage that plots are arranged systematically and there is no randomization of treatments. It is therefore important to ensure that such factors as water supply, access to fields, etc., are not always arranged in the same relative position on the different sites, otherwise there may be a repeated gradation from one treatment to the next. It is unlikely that such factors as variations in soil type or slope will always show the same relative variation from one site to the next. Obviously this type of systematic lay-out should be avoided if one treatment is likely seriously to affect the one next to it in the field. However, as the differences to be measured will normally be fairly large and the experiment will normally be based on a controlled experiment at the Grassland Research Institute, it is considered that the advantages the simplification of lay-out has on a commercial farm outweigh the practical disadvantages which may arise from more complicated lay-outs. If the individual areas to be treated by the farmer (e.g. grazing and fertilizing) were each 4 acres in Fig. 12, the size of the small treatment plots would be 0.5 acres each. This is the smallest size desirable when the managements are applied on a farm scale; plots of a smaller size are more susceptible to errors arising from faulty fertilizer distribution, etc. Experiments involving grazing managements create problems of fencing. If cattle are used, electric fencing is most useful, but with long-term experiments one "break-through" may spoil a whole series of results, and it is worthwhile erecting a semi-permanent fence of wooden posts connected by one or two electrified strands of barbed wire.

It is, of course, desirable to have at least two blocks at any one centre but grassland experiments involving 'farmer' treatments usually raise problems of fencing and watering. To have more than one block will probably require the splitting of the grazing animals into at least two groups and this is often particularly difficult if dairy cows are involved. In order

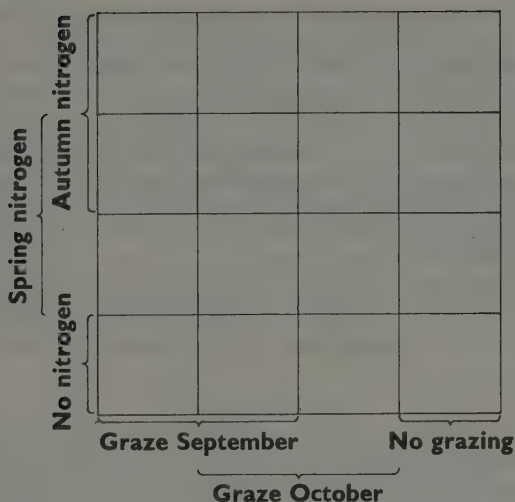


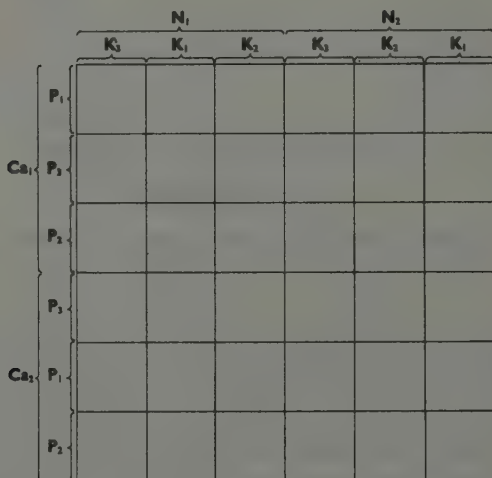
FIG. 12. Arrangement of fertilizer and grazing managements to simplify application of treatments on a farm trial.

to obtain maximum information from grassland experiments it is frequently necessary to run long-term experiments and this accentuates these problems so that it is often only possible to operate one block per centre. Replication is then obtained by having a number of centres in one particular environment. It should be remembered that this type of experiment is primarily concerned with examining wide differences or confirming results already obtained under controlled conditions at the research institute.

It may be necessary to examine a wide range of treatments (e.g. levels of fertilizer or grass varieties) which can be laid out in small plots so that the appropriate managements can be applied by hand. The fact that the plots are small and that technicians apply the treatments, allows the use of normal randomization and replication. However, because of the small size and relatively large number of plots such an experimental area will normally have to be grazed as a single unit and this can cause differential grazing between treatments. When results are required from uniform grazing conditions the selection of treatments may have to be limited. Thus, when examining a collection of grass varieties only those with similar characteristics should be compared at any one site, also the plots should be in an area sown to a similar mixture to avoid excessive over- or under-grazing. The lay-out of the plots in the field must be related to the grazing management adopted by the farmer. Ideally all plots should be grazed at once but if strip-grazing is practised and only part of the area

may be grazed at any one time, the plots must be sited so that the electric fence is moved simultaneously across all plots within a block.

Individual areas (e.g. a specific soil type) may require special investigation. The problems will often be peculiar to the individual areas and most of the experimentation may have to be carried out *in situ*. One must first define these problems and it may be necessary to lay down a wide range of treatments in order to obtain preliminary information. Fig. 13 shows a lay-out adopted on a problem soil area which proved extremely useful in giving a guide to future investigations. The criss-crossing of treatments gives a very large number of treatments and interactions can readily be identified.



N₁ — N₂ — levels of nitrogeaneous fertilizer

K₁ — K₃ — levels of potash fertilizer

P₁ — P₃ — levels of phosphate fertilizer

Ca₁ — Ca₂ — levels of lime application

FIG. 13. Criss-crossing of fertilizer treatment on an observational trial.

CHAPTER 19

MEASUREMENTS

Observations

The value of notes and records cannot be over-emphasized. In the examination of a wide range of treatments aimed at defining the problems on a specific soil type, observational notes have been of major importance and accurate, frequent scoring has proved essential. Scoring measurements have also been adopted for assessing the value of grass varieties under practical farming conditions. The varieties are sown in small plots and frequent scoring observations are made; no more than five classifications are given because, at any one time, the object is to record major differences only.

Herbage sampling

The basic principles in herbage sampling techniques are similar to those in use at the Institute. Where possible the use of the motor scythe [2]* is favoured for the following reasons: (i) it is of robust construction and, when properly maintained, is trouble-free in action; (ii) large areas can conveniently be sampled. This is desirable when operating on a field scale; (iii) the cut material may be sub-sampled for botanical analyses; (iv) it can easily be transported in a motor van which has been fitted with ramps. It is very mobile in the field, an essential point when large distances in the field may have to be traversed; (v) the height of the cut is uniform and relatively insensitive to minor variation in the level of the ground surface.

The motor scythe has proved particularly useful when estimating the amount of herbage present under rotational systems of grazing with cattle. The grass is normally sufficiently well grown for sampling and the amount left is similar to that left by the cattle.

When sampling young, leafy herbage the motor scythe leaves a much higher proportion of uncut material and in some experiments on the production of early grass the herbage has been cut first by a motor scythe and then by a cylinder mower. The amount left for collection by the cylinder mower was fairly consistent at any sampling period within an experiment or on any particular site whilst the amount collected by the motor scythe recorded most of the changes due to treatment. The shorter the grass, the greater was the proportion picked up by the cylinder mower (Table 5). In the same experiment, no cases were recorded where the total yields obtained by both motor scythe and cylinder mower were in a different order from those obtained by the motor scythe alone. When the herbage for sampling yields at least 800 lb of herbage per acre the motor scythe is considered a suitable machine for this purpose, particularly on field plots when large sample cuts can then be obtained. A major difficulty with

*Figures in [] refer to Appendix 2.

Table 5.

Comparison of yields (lb dry matter/acre) obtained by motor scythe alone or by motor scythe plus cylinder mower

Expt	Cutting technique	Treatment									S.E. of mean
		1	2	3	4	5	6	7	8	9	
H.65	Motor scythe	200	380	640	220	410	530	290	480	650	±47
	Motor scythe plus cylinder mower	720	1000	1280	730	1030	1160	840	1130	1360	±66
H.140	Motor scythe	980	1130	2270	2300						±67
	Motor scythe plus cylinder mower	1270	1430	2570	2610						±69

cylinder mowers is that they will not normally stand up to the harsher sampling conditions experienced on commercial farms. Hedge trimmers and hand shears are generally not used because of the relatively small areas which can be sampled by these tools.

The green weights of samples are recorded in the field and sub-samples transported to Hurley for dry-matter- and crude-protein determinations. In order to prevent changes during transportation due to respiration, etc. the samples are frozen in insulated containers [22]. The external dimensions are $18 \times 24 \times 19$ in. and the internal $13 \times 19 \times 16$ in. The herbage samples are frozen by the use of 28-lb blocks of solid carbon dioxide. One block is sufficient to freeze a container full of samples; the time required for freezing the samples is extremely short and is normally less than 30 minutes. Solid carbon dioxide has to be replenished after 36–48 hours but an additional 28-lb block will last a further 48–72 hours. In practice it is ordered from the manufacturers in advance and collected from one of their many depots throughout the country. Normally 20–30 samples, each of 300–800 g, are stored in each container.

The following schedule is that normally followed in the sampling of field-scale plots (approximately 0.5 acre in area) in which the herbage is at least 6 in. long:

- (i) Five herbage cuts, each 6 yd in length, taken at random with the motor scythe,
- (ii) Herbage from the 6 cuts bulked and weighed on a tripod,
- (iii) Two sub-samples each of approximately 800 g taken, placed in polythene bags and immediately tied,
- (iv) Sub-samples placed in insulation boxes for transportation to Hurley,
- (v) At the Institute the whole sub-sample, plus polythene bags (standard weight of bag being known), is weighed. The herbage is removed, dried and again weighed. After grinding, two samples are taken from each sub-sample for crude-protein analysis,

- (vi) A further two sub-samples may be taken from the bulked field sample for botanical analysis.

The accuracy achieved by this method of field bulking and sub-sampling is reasonable. Thus in an experiment with a winter grass in which the herbage yields ranged from 100 to 2500 lb dry matter per acre and in which the herbage on a plot was often very heterogeneous, the average coefficient of variation between sub-samples for both dry-matter- and crude-protein-determinations was approximately 4%.

The full equipment of the mobile sampling unit used for herbage and botanical assessments is shown in Plate 23 and consists of:

1. A van complete with luggage rack,
2. Motor scythe,
3. Two ramps for motor scythe,
4. Metal tripod,
5. Two canvas tares for collecting samples,
6. Spring balance for weighing 50 lb,
7. Rakes,
8. Marker for measuring length of cut,
9. Polythene bags, labels, string,
10. Insulated boxes—up to 6 can be carried on the luggage rack,
11. Canvas cover for luggage rack held in place by clip-on elastic expanders.

Using this equipment and technique a team of two can visit a farm and sample 12 plots (each 0.5–1 acre in size) within 3 hours.

When continuous records of herbage production over extended periods are required and the plots are likely to be grazed, field cages are used to protect the herbage (Cowlshaw, 1951; Brown, 1954). Whenever practicable the cages are placed on the fields immediately before grazing so that the periods of grass growth under the cages are reduced to a minimum. The cages are moved to a fresh pre-treatment site after each sampling. The area covered by each cage is 9×4 ft and at each sampling a cut 3 ft long is taken the full length of the cage. When operating on a field scale, 10 cages are usually used in each treatment. At the beginning of one experiment in which the plot size was 4 acres, 10 cages per plot were used and the coefficient of variation between the individual sample cuts was approximately 12%. Plates 24 and 25 show a simple trailer which is used for moving cages around experimental fields. The frame of the trailer is made from tubular steel piping with a diameter of 1.5 in. The wheels have 16×4 in. heavy-duty tyres and 10 cages (each weighing approximately 50 lb) can be carried at once. Two dowel pins and a locking nut at point 'A' join the towing-bar section of the trailer to the axle. The locking handle has a handle welded on for easy tightening and the two portions of the trailer may be easily separated for transportation.

Animal measurements

In order to assess live-weight production from grassland a portable weighbridge [7] (Plates 26 and 27) is used. The framework of this weighbridge is of tubular metal construction with a wooden floor. In order to

prevent stock from placing their head or limbs through the framework, metal panels have been made to enclose fully the sides of the weighing platform. The dial of the weighbridge is operated by a spring balance and is graduated into three different scales: 20 cwt in 7-lb divisions, 2200 lb in 10-lb divisions, and 1000 kg in 5-kg divisions.

Two retractable wheels are fixed to the frame of the weighbridge which allows easy manoeuvring, but it is necessary to ensure by the use of a spirit level and wooden wedges that the machine is level before weighing begins. Weights of 56 lb each are also carried to check the accuracy of the weighbridge before and during the weighing. A special trailer has been made at the Institute for transporting the weighbridge, (Plates 26 and 27). The weighbridge is loaded and unloaded by means of two ramps and a hand winch. Retractable jacks at points A and B hold the trailer steady whilst the weighbridge is loaded or unloaded. This trailer has the added advantage that it may be used for other transportation when not carrying the weighbridge.

This portable weighbridge was compared with a semi-permanent weighbridge [5]. Forty cattle each weighing between 4 and 7 cwt were used and the average difference in weight recorded by the two machines was less than 1 lb per animal. The maximum difference measured for any single animal was 6 lb.

One of the major errors involved in weighing livestock is the variation in the amount of 'fill' (see p. 71). In order to reduce this variable to a minimum, the cattle are weighed at a constant time (10–11 a.m.). To record changes in live weight on farms the minimum period for grazing any treatment is considered to be one month. Animals may also enter experiments after grazing very dissimilar herbage; and, if animal production from a particular sward is required, the factors affecting fill should be as similar as possible at the first and last weighings. Therefore animals are first weighed after they have been grazing the plots for two or three days and last weighed three or four days before it is estimated that they will have finished grazing the plots. This last weighing ensures that they are weighed before the supply of herbage becomes a serious limiting factor, as may happen if the amount of herbage available is misjudged and the animals are kept on the field until the weighbridge arrives.

Extension trials often require the collection of information on grazing records. These records must be as simple as possible if they are to be kept by the farmer, but on the other hand sufficient information must be collected to provide data for the correct interpretation of results. The minimum records which can normally be accepted are: class of stock, number of grazing days, the amount of supplementary food fed. When dairy cows are used, milk records are also desirable.

The records used in the extension trials of the Institute are modifications of those recommended by the British Grassland Society (Barker, A. S. *et al.*, 1955). Other systems of pasture recording are discussed by Brown (1954).

PART VI

SPECIAL LABORATORY EQUIPMENT AND ITS USE

CHAPTER 20

DETERMINATION OF THE DRY-MATTER CONTENT OF HERBAGE, FAECES AND SILAGE

Yields in grassland investigations are usually expressed on a dry-matter basis, so as to eliminate the effects of the wide variation in moisture content of fresh-weight yields. Dry matter is generally defined as the constant weight a sample attains when heated at 100°C (occasionally 105°C). This definition, while suitable for inert materials such as sand, suffers from three main deficiencies when applied to biological materials. (i) In these materials water is present in various states ranging from adventitious moisture as rain or dew, through water present in cell-sap, to water present in various physico-chemical and chemical combinations. The extent of the displacement, by heating, of water in these latter states depends on the heat-stability of the various chemical groupings present (sugars may lose water and form anhydro-sugars) so that the dry-matter content of herbage can never have the same precise meaning as that of an inert material. (ii) Biological materials contain active respiratory enzymic systems which continue to function during the early stages of drying; in fact, as the material heats up, activity will be enhanced until it is halted either by denaturation of the enzymes or by desiccation. Besides altering the chemical composition of the material, such activity will lead to loss of dry matter. (iii) Most biological materials contain organic compounds which are volatile at 100°C and which are therefore lost on drying. This leads to an underestimation of dry-matter content, which may be particularly serious when faeces or silage are dried.

Thus it must be accepted that any method adopted for the determination of the dry-matter content of biological materials must involve compromise. Low-temperature drying is slow, and, while it will reduce losses of volatile or heat-labile compounds, losses due to prolonged enzymic activity are likely to be large. More rapid drying at 100°C will reduce these losses, but may lead to other losses of volatile or heat-labile components. Where it is desirable that changes in chemical composition should be kept to a minimum during drying, freeze-drying has been employed (Davies *et al.*, 1948) but this cannot be used to determine dry-matter content as samples do not come to a standard end-point: the more volatile organic compounds may also be removed during the process.

A further important consideration is that the method adopted should be applicable to adequate numbers of samples of sufficient size to satisfy the requirements of sampling accuracy discussed in Part I. In general the more samples that are dried in a particular oven, and the larger their size, the more slowly do they dry, and therefore the greater are the respiratory losses during drying likely to be. This problem can be reduced by increasing the size of drying oven, but it then becomes more difficult to ensure a uniform final temperature (essential to the definition of 'dry matter') within the oven. Thus the competing claims of number and size of samples, and rapidity of drying, are also seen to involve a compromise.

Early in the present investigations a study of the drying ovens then available revealed various deficiencies, among which the most important were: (i) inadequate capacity, (ii) no through-draught, so that, even at high temperatures, samples dried slowly, (iii) where a through-draught was employed the drying air passed through a series of samples, so that the later samples dried much more slowly, and at a lower temperature,

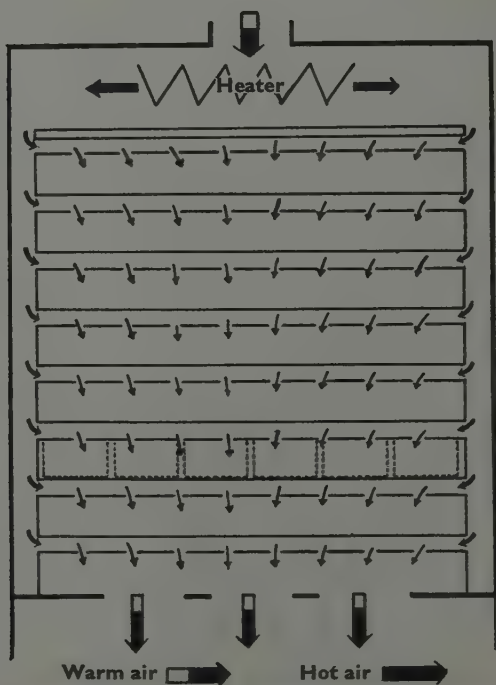


FIG. 14. Drying oven. Hot air is distributed to all parts of the oven.

than the earlier samples, (iv) drying temperatures were frequently well below 100°C, (v) inlet temperature was well above 100°C, so that samples near the inlet were scorched. No one oven, of course, exhibited all these faults! As a result of these studies an oven with the following characteristics was designed and built: (a) a capacity of up to 96×400 -g samples of fresh herbage, (b) accurate thermostatic control of inlet air temperature at 100°C, (c) rapid air-flow to ensure quick removal of water-vapour, (d) *fresh* hot air ducted into every part of the oven to ensure uniform drying and final uniform temperature, (e) suitability for economic production. Based on this prototype design a drying oven was developed which is now widely used in agricultural laboratories [26]*. In this oven (Fig. 14) air is electrically heated to 100°C in the top compartment and then blown into the drying chamber (capacity 50 cu. ft) through holes in the undersides of 32 ducts, which themselves act as the shelves on which the trays of samples rest. After passing through the samples, the air with its acquired water vapour is led up the back of the oven, a proportion rejected and the remainder, with further fresh air, passed through the heater bank and into the drying chamber again. Tests (*Laboratory Practice*, 1956) have shown that, except in the earliest stages of drying, the temperature varies little between different parts of the oven: this results partly from the system of multiple ducting, and also from the use of a 'plenum' system, in which hot air is forced *down* the oven.

In many tests it was found that all the samples in a full load came to constant weight in less than 8 hours, but it was realized that, even during this period, enzymic losses were occurring since material in the centre of the herbage samples remained below 50°C for at least an hour. To study the magnitude of these losses, an oven using high-frequency dielectric heating, which would dry a 100-g sample of herbage in 15 min with a drying temperature below 60°C, was constructed. Using this as a 'standard' it was found that between 1% and 2% of the dry matter in samples dried in the newly designed oven was being lost (Raymond & Harris, 1954).† This was too high to be accepted in many studies and methods for reducing drying times were investigated. It was found desirable: (a) to pack samples loosely in the largest possible drying trays (the standard drying tray [27], dimensions $7 \times 18 \times 3.5$ in., with wire-mesh floor, should not contain more than 400 g fresh herbage, and samples larger than this are dried in special trays $13 \times 18 \times 3.5$ in.), (b) to avoid fully loading the oven (80 samples were found to dry much more rapidly than the full load of 96 samples), (c) to space these samples as uniformly as possible within the oven, (d) always to pre-heat the oven to 100°C before loading samples, (e) to avoid introducing further wet samples, once samples have started to dry. With these precautions, tests have shown dry-matter losses to be under 0.5%: the most effective measure is the reduction in total load of samples to be dried at one time. However, in view of the empirical nature of the concept of dry matter in biological samples noted earlier, complete elimination of drying losses seems impracticable, and in

*Figures in [] refer to Appendix 2

† Up to 10% loss of dry matter had been recorded in one oven tested.

fact may be undesirable if it is attempted at the expense of the number or size of samples dried, for sampling errors are generally of much larger magnitude than drying errors.

Herbage. Materials such as herbage, when completely dried, rapidly pick up moisture, and dried samples taken from the oven must be weighed as rapidly as possible once they have cooled. They should then be milled while still crisp, as milling equipment then operates much more efficiently (p. 139).

Faeces. Faeces dry much more slowly than herbage containing the same percentage of moisture, because of the formation of an outer 'crust' which impedes diffusion of water vapour from the centre of the material. Therefore faeces should be broken up and spread out as much as possible before drying: even when this is done wet faeces (12% D.M. content) may take up to 24 hr at 100°C to reach constant weight. Losses of dry matter from bacterial action during the early stages of drying, and from loss of volatile constituents, especially amine compounds, are likely when drying faeces. However, with care these should be under 2% of the dry matter present. Where the nitrogen content of faeces is being studied, however, the loss of volatile nitrogenous constituents may be appreciable and in accurate studies nitrogen determinations should be made on fresh faeces (p. 142).

Silage. The content of volatile constituents, especially volatile fatty acids, in silage may be as high as 10% and the majority of these will be lost during drying at 100°C. These constituents are of nutritional value to the ruminant. This must mean that the dry-matter content of silage as a ruminant feed will be underestimated by the amount of these volatile losses (failure to allow for these losses probably accounts for an appreciable proportion of the losses of dry matter which have been reported in investigations of the ensilage process). A technique for drying a 100-g sample of silage in a flask immersed in boiling water and condensing the volatile fraction, has been tested. The contents of volatile fatty acids and bases in the condensate can be determined and the dry weight of the silage corrected for these losses (see also McDonald and Dewar, 1960). However, a more practicable method appears to be the determination of the energy content of fresh silage in terms of Kcal (p. 147).

CHAPTER 21

PREPARATION OF DRIED SAMPLES FOR CHEMICAL ANALYSIS

In the great majority of studies, drying herbage or faeces in the drying oven [26] is quite adequate for subsequent chemical analyses. The dried materials must, however, first be milled or powdered to produce a material suitable for sub-sampling. Milling is generally carried out in either a hammer mill or a shear-mill. In the former, which is most widely used in the United Kingdom, the dried sample is subjected to repeated beating until the particles are small enough to pass through a grid into a cloth bag [32]. Careful study of this process has shown that in general it is carried out most unsatisfactorily. Milling is a dusty, noisy and unpleasant operation, and, because of this, it tends to be carried out as quickly as possible, and frequently carelessly. Often only a proportion of a sample is milled, which leads to sampling errors; the cloth bags are seldom completely emptied or cleaned before the next sample is milled; and sampling of the milled material may not be carried out carefully. In addition, recent work has shown (Bailey *et al.*, 1957) that there may be a differential loss of high-nitrogen-content particles through the pores of the bag itself and through channels where the bag is clipped under the mill, because air pressure develops inside the bag during milling. This loss is due to dried cell-contents shattering into much smaller fragments than the fibrous structures of the plant: it is particularly serious when freeze-dried materials are milled, for, in these, the cell contents do not coagulate as they do during heat drying.

These losses would appear less likely in shear-mills, which do not depend on a through air-flow for their operation. However, shear-mills grind more slowly than hammer mills, and, after a sample is ground there is usually a residue of unmilled fibre inside the mill which must be removed and mixed with the milled fraction. Both these factors are likely to lead to careless milling of samples.

Thus a system of hammer-milling that reduces to a minimum the loss of fine particles (and at the same time the dustiness which is the most unpleasant feature of grinding) and allows good sampling of the milled material, was investigated. As noted above, the hammer mill used [32] will not operate if its through air-flow is completely stopped, but it was found that the area of cloth through which air escapes can be greatly reduced from that in the usual collection bag. A metal box was constructed, with nylon cloth panels in the front and back walls and with a rubber gasket on the top flange (Fig. 15). For quick positioning and removal the box is raised on a cam-action lever which is adjusted so that the top gasket presses firmly against the base of the mill, thus avoiding any air-leakage there. While the rate of grinding is undoubtedly reduced as a result of restricting the air-flow, the size of nylon panels used gives a reasonable grinding rate with minimal losses of fine particles. To stop stray dust

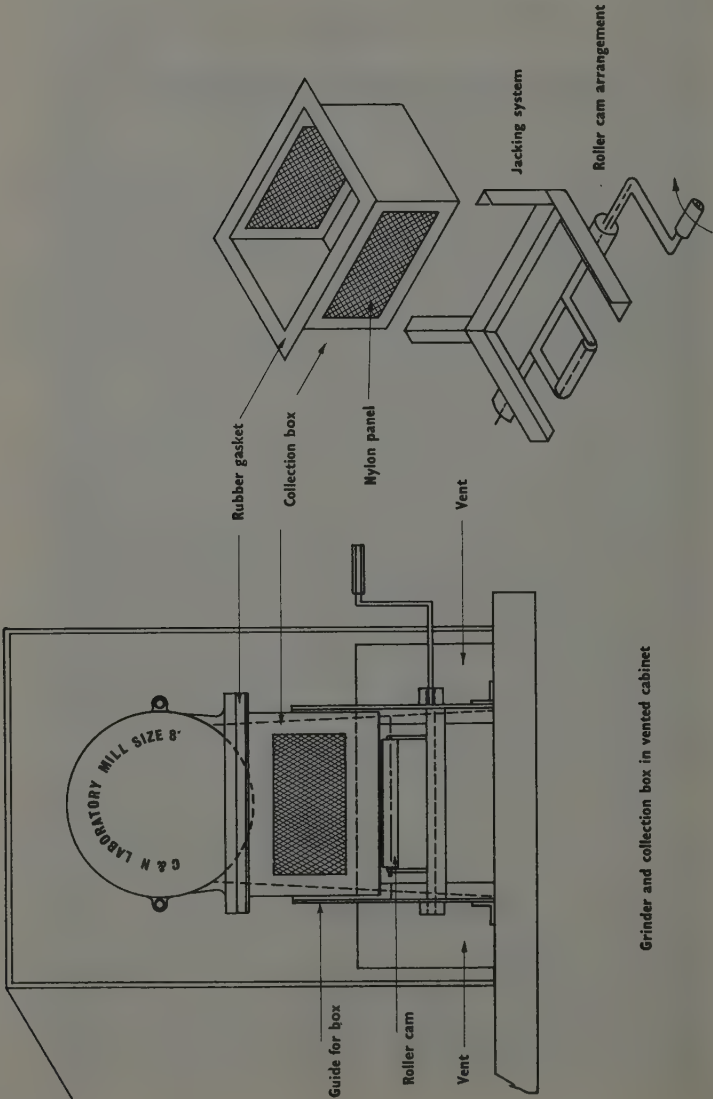


FIG. 15. Detail of laboratory mill, sample-collection box, and jacking system for positioning box tightly against base of mill.

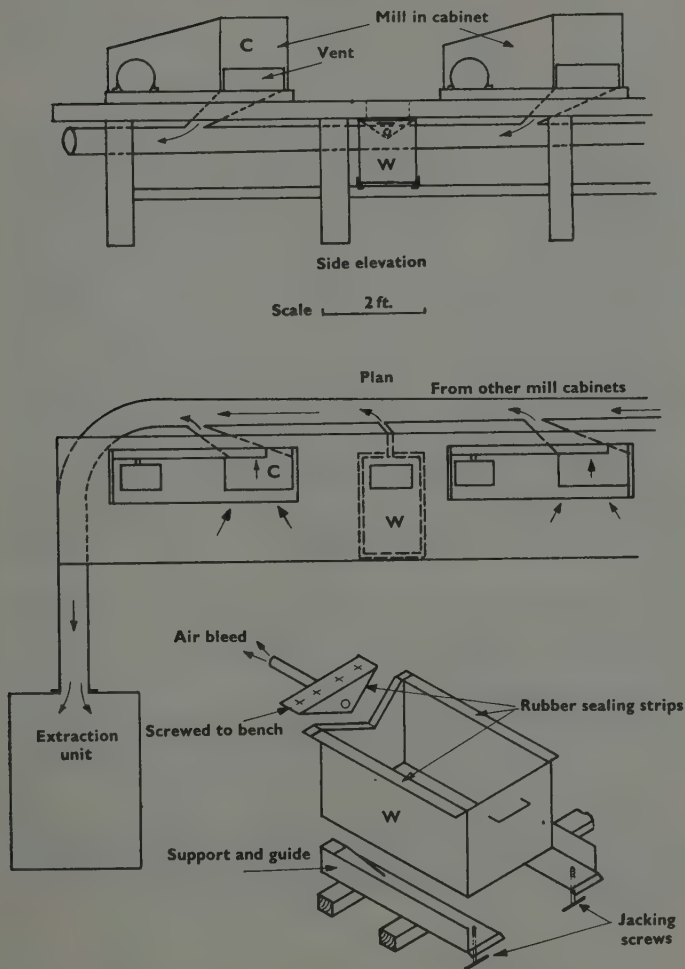


FIG. 16. Dust extraction system for laboratory mills: each mill is enclosed inside a cabinet, C, from which air is extracted. Surplus milled material is tipped into ventilated waste hopper, W.

entering the grinder room, the mill and collection box are enclosed within a hood from which air is drawn by a fan through filter cloths and back into the room. (The equipment used [28] gives very efficient ventilation at three grinder points: such an 'internal' system is preferable to simple ventilation to the outside of the building because it greatly reduces the space-heating load in winter.)

Once a sample is milled, the mill is switched off and allowed to stop. The box is then removed, inverted and placed with the gasket firmly on a sheet of white paper, and tapped smartly to dislodge any particles adhering to the box or nylon panels. The sample is then mixed thoroughly but gently, the required amount of sub-sample taken, and the surplus tipped into a waste hopper on the bench, which is also ventilated by the dust-extraction system to avoid any dust flying back into the room. (A diagram of this system is shown in Fig. 16.) With samples having a very high nitrogen content it is sometimes found that a small amount of the sample sticks to the inside of the mill. This may need brushing out, but this is generally only necessary where (a) very small samples, e.g. 20 g of dry matter, are being milled, (b) consecutive samples are likely to vary markedly in nitrogen content. However, the need for cleaning the mill between samples should always be considered in relation to the type and size of samples being milled and to the precision desired in the particular investigation.

For empirical analyses, such as crude fibre, the particle size of the milled material is important. Thus the size of holes in the grid of the mill must be standardized (in our own studies a 0.8-mm grid is used) and, in addition, the grid must be regularly replaced as the holes slowly enlarge because of abrasion from sand, fencing wire, etc. in the samples being milled.

Where the sample of herbage or faeces to be milled is less than 20 g it is preferable to mill it in a small laboratory mill [32] because of the loss of fine particles if the larger mill and collection box are not completely cleaned between samples.

As noted above, differential losses from freeze-dried samples are likely to be particularly serious and, in addition, these samples are generally small. For these reasons freeze-dried samples are best milled in a ball-mill, in which the sample is enclosed with a charge of stone balls inside a porcelain jar which is rotated about its long axis at about 1 rev./sec.

Milled herbage and faeces samples still contain a noticeable range of particle sizes and larger particles are often found to be at the top of the milled material in sample containers. The material must be carefully but gently stirred before a representative sample can be taken for chemical analysis.

CHAPTER 22

PREPARATION OF SAMPLES OF FRESH HERBAGE, FAECES AND SILAGE FOR CHEMICAL ANALYSIS

As noted earlier, biological materials undergo chemical changes during oven-drying because of the direct effect of high temperature, or of enzymic or bacterial changes during the early stages of drying, or of loss of volatile constituents. Thus the precise study of the chemical composition of these materials requires that analyses should be made on fresh, undried samples. However, chemical analyses generally require only small samples of material (e.g. crude-protein analysis is carried out on 4–6 g of fresh herbage or faeces) and accurate sub-sampling of such small amounts from a larger bulk sample is most difficult. The difficulties have been overcome by taking a larger preliminary sub-sample, mixing this very thoroughly and then taking the final sub-sample from this mix. In the case of herbage and of faeces the bulk sample is first mixed as well as possible and a 150-g (approx.) sub-sample taken. This is thoroughly macerated with 150 ml of water and 5 ml toluene in a bottom-drive macerator [25]. Faeces samples give a homogeneous cream which can be accurately sub-sampled. Herbage samples, however, give a 2-phase mix, with fibres floating in a dilute solution of cell contents, which is very difficult to sample. To overcome this, 5 g of bentonite are added before maceration. This absorbs the aqueous phase and the fibre disperses uniformly in the resulting paste, allowing accurate sub-sampling.

Herbage. With herbage the initial fresh weight of herbage, the weights of water and toluene added and the dry weight of the bentonite added must all be known in order to allow the chemical composition of the dry matter in the fresh herbage to be calculated from the composition and dry-matter content of the macerate. This technique has been particularly useful for preparing fresh samples for Kjeldahl nitrogen determinations. It has been found convenient to weigh the macerate sub-sample into a flat-bottomed glass tube which will pass down the neck of the Kjeldahl flask, so avoiding macerate adhering to the neck.

Faeces. From faeces macerates, sub-samples are weighed for chemical analysis, and at the same time other samples (approx. 10 g) are taken for determination of the dry-matter content of the macerate: these are dried to constant weight in an oven at 100°C; the toluene reduces bacterial losses during handling and drying.

Silage. Silage can be treated in exactly the same way as herbage samples, but it has generally been found adequate to take analytical sub-samples directly from the comminuted material from a macerator mill (see p. 147). Mincing machines, used for the same purpose, express juice which is difficult to re-mix with the minced sample; they have proved less satisfactory.

CHAPTER 23

DETERMINATION OF THE GROSS CALORIFIC VALUE OF HERBAGE, FAECES, URINE AND SILAGE

The calorific (energy) values of feeds, faeces and urine from digestibility experiments are frequently determined. The method for measuring calorific value, and the special problems arising in the sampling and preparation of these materials for energy determinations, are discussed.

Method for measuring calorific value

The calorific value of a sample is determined by igniting it in a bomb calorimeter and comparing the rise in temperature of the water in the inner (bomb) vessel with that produced by the combustion of a material of known calorific value (generally benzoic acid). The transfer of heat from the sample burning within the bomb to the water usually takes 10–12 min: during this period the water is at a temperature above that of its surroundings and is losing heat. A correction for this must be made by making the surroundings effectively isothermal and recording the rate of fall of temperature of the water in the inner vessel after it has reached a maximum. From this the maximum temperature which would have been reached had there been no heat loss to the surroundings, and thus the 'true' temperature rise of the inner vessel, can be calculated.

Calorimeter systems

While, with careful use, the isothermal bomb calorimeter gives highly accurate results, it is slow and laborious: a single determination occupies over half an hour and during much of this time the operator has to record temperatures every half-minute. Various workers have therefore proposed adiabatic systems, in which there is no temperature differential between the inner vessel and the water in the outer vessel, so that the observed temperature rise of the inner vessel is also the 'true' temperature rise. Most of the systems employed have been manually operated, but recently a fully automatic adiabatic bomb calorimeter has been developed at this Institute, and has now been used for many hundreds of energy determinations.

The automatic adiabatic bomb calorimeter

Particular emphasis was laid on developing a system which could be used for modifying existing isothermal bomb calorimeters. The basic requirement is for an automatic device to raise the temperature of the water in the outer jacket (which in the isothermal system is assumed to remain at constant temperature) at exactly the same rate as the water in the inner vessel is heated by combustion of the sample in the bomb. There is then no net gain or loss of heat from the inner vessel system to

its surroundings. This has been achieved by locating temperature-sensitive elements (thermistors) in the water in the inner and outer vessels; these thermistors are connected in two arms of a sensitive a.c. Wheatstone bridge and any rise in temperature of the inner thermistor unbalances this bridge. This operates relays controlling heating elements in the water in the outer vessel which rapidly raise the temperature to that of the water in the inner vessel. If thorough stirring of the water in both vessels is ensured, a temperature differential of less than 0.1°C during even the most rapid heating period can be achieved. To increase precision, the inner vessel is closed with a hollow lid through which water from the outer vessel is pumped. The system will maintain water in the inner vessel at 5°C above ambient temperature for 30 min, without any measurable fall in temperature.

This equipment has been described, with details of the control circuit, calorimeter modifications, etc. (Raymond *et al.*, 1957). The main improvement now advised is the use of a hollow lid to the inner vessel through which water from the outer jacket is passed.

Method of use. A known weight of sample is placed in the dish. This is placed inside the bomb with the ignition wire fitted, and the bomb sealed and then charged with oxygen at 25-atm. pressure. The bomb is then immersed in the water in the inner vessel (a fixed weight of water is used), the electric firing leads are attached and the adiabatic lid fitted. There are 4 holes in this lid through which pass the stirring mechanism, thermometer (in the present model two thermometers are used, graduated to 0.01°C and read to 0.002°C and giving a total temperature range of 15° – 24°C) and one thermistor element. The water in the outer vessel, in which the second thermistor is located, is cooled slightly by adding tap water. As soon as the temperature in the inner vessel becomes effectively constant the adiabatic control is switched on and rapidly raises the temperature of the water in the outer vessel and lid to that of the inner vessel. The temperature of the latter is then recorded (it should be changing by less than 0.002°C per min at this stage) and the sample ignited electrically. After a time lag of some 30 sec the temperature of the water in the inner vessel begins to rise, and almost immediately the control starts heating the water in the outer vessel, switching itself off and on as the temperature of this water tends to rise above, and then lag behind, that in the inner vessel. Maximum temperature is reached in 13 min with this equipment. The temperatures are recorded after 13 and 14 min and should agree to within 0.002°C . The control is then switched off and the bomb removed, emptied and re-charged with another sample for a further determination. The water in the inner vessel is only changed after 3–4 determinations, as the equipment can safely be used well above ambient temperature; however, the inner vessel must always be weighed and any loss of weight resulting from removal of water adhering to the bomb made good before each fresh determination.

During the 'chief period' of the determination the operator is free to carry out the preparation and weighing of other samples; alternatively, if large numbers of determinations have to be made, one operator can

readily supervise two calorimeters at the same time. In our own studies one operator, using a single calorimeter, has made 12 determinations per day, including the preparation of samples, over periods of several weeks. In routine use the coefficients of variation of a single estimate of the energy content of dried herbage or faeces have been 0.29% and 0.28%, respectively. The coefficient of variation of estimate of the energy content of urine has been much higher, at 2.92%, because this value is obtained by difference (see p. 147). The conversion described (*ibid*) can be carried out in a workshop equipped with a few machine tools and facilities for assembling electronic components. Alternatively, commercial equipment is now available [24].

The preparation of samples for bomb calorimetry

As noted in the discussion of the determination of dry matter, samples of herbage, faeces and urine are biologically active and tend to lose dry matter by respiration and bacterial action unless dried rapidly. In addition silage, faeces and urine are likely to suffer losses of volatile components during heat drying.*

Herbage. With adequate care herbage should lose a negligible proportion of its dry-matter content during oven drying (see p. 137). The dried material is then milled and the milled material allowed to come to equilibrium with atmospheric moisture. 1.2–1.4 g of this material, giving a heat release of approximately 6000 gcal, similar to that released from the benzoic acid used for calibrating the calorimeter (see p. 144) is then pelleted and the pellets weighed immediately. At the same time a second sample is taken and oven-dried at 100°C to estimate the moisture content of the pelleted sample. This method of determining the amount of dry matter in the pellet has been adopted because: (a) during pelleting of oven-dried samples appreciable amounts of moisture may be absorbed and (b) a pellet made from milled grass equilibrated in air tends to break up if it is subsequently oven-dried to determine its dry-matter content.

Faeces. The energy content of faeces can be determined either on milled, dried faeces exactly as described above for herbage, or on faeces which have been dried at low temperature *in vacuo* to reduce losses of volatile constituents. Colovos *et al.*, (1957) have suggested that heat-drying of faeces may lead to considerable losses of energy: however, as noted in the discussion on the determination of the dry-matter content of faeces (p. 138), low-temperature drying of faeces, while it may reduce losses of volatile constituents, may lead to other losses resulting from prolonged bacterial activity. Thus, in all but the most precise studies, a drying method which allows a fairly large sample of faeces to be dried (so reducing sampling errors) with minimal losses seems desirable, both for the determination of the dry- and organic-matter content and of the energy content of faeces. As noted on p. 138, rapid oven-drying at 100°C of 500- to 1000-g lots of faeces, well spread out in perforated trays, should reduce losses to

*Colleagues at the Hannah Dairy Research Institute, Scotland, gave valuable advice on the preparation of samples, and on acid corrections (p. 148).

below 2%; these samples can then be milled and used for the determination of energy content (see *Herbage*, above).

The energy content of 'fresh' faeces, where required, is determined by accurately weighing 4-6 g of well-mixed faeces into the crucible in which the sample is to be ignited in the bomb. The crucible and contents are then placed in a vacuum desiccator over P_2O_5 in a refrigerator at 5°C. Most samples of faeces are dry enough to ignite after 24 hours' drying. Comparison of the energy contents of several faeces' samples, dried in this way, with those of samples of the same faeces dried in 1000-g lots in an oven at 100°C showed an average loss of 1.2% in energy content during oven-drying, both energy contents being expressed as Kcal per gram of fresh faeces. This difference is very similar to the average loss of dry-matter found during the drying of these large samples, indicating that there is no loss of higher-energy constituents during oven-drying.

Urine. When urine is dried it forms a sticky layer on the base of the crucible which will not ignite in the bomb. The method used here is to absorb approx. 10 g of urine in a block (14×16 mm) of filter paper cellulose of known energy content. The exact weight of urine is determined by weighing the crucible containing the block before and after the urine is added. The sample is then dried over P_2O_5 in a vacuum desiccator at room temperature. After 48 hours the sample is sufficiently dry to burn inside the bomb. From the total heat release, measured with the calorimeter, the heat produced by combustion of the cellulose block is subtracted to give the energy of combustion of the urine. The combustion energy of the block is estimated from control determinations on similar blocks, each block being weighed to allow for slight variations between their dry weights.

The total error of the determination is applied to the estimate of the combustion energy of the urine, which is generally only about 15% of that of the urine plus block. This accounts for the high coefficient of variation of a single estimate of the energy content of urine, 2.92%, noted above. However, the effect of this on the determination of metabolizable energy is not as serious as might appear because urine energy is usually only some 10% of the metabolizable energy of a feed.

Silage. As noted above, volatile constituents are lost when silage is oven-dried, resulting in underestimation of the dry matter and energy contents of fresh silages. A technique for measuring the energy content per gram of fresh silage has been developed and in *experimental* work it is suggested that losses during ensilage, amounts of silage fed, etc., should be expressed in Kcal, rather than in g dry matter, as at present.

For this technique small representative samples of silage (approx. 5 g) are required. Sub-samples of approx. 300 g, similar to those usually taken for dry-matter and chemical determinations, are taken from the well-mixed bulk of silage. Each of these is then macerated separately in a mill [33] which consists of a totally enclosed chamber composed of two hinged hemispheres, inside which the sample is macerated by rotating knives. No moisture is lost during the 1-min mincing time and the sample can be completely removed when the front hemisphere is swung aside. It

has been found that a 300-g sample of silage can be accurately sub-sampled in this way (e.g. nitrogen determinations on 5-g sub-samples have shown a coefficient of variation of only 0.5%) so that the main effort is required in sampling the original bulk of silage. With long silage this is markedly improved by chaffing the silage before sampling.

Each sub-sample of silage from the macerator is ignited inside the adiabatic bomb calorimeter, a weighed amount of benzoic acid (approx. 1.0 g) of known energy content being used as a primer. This is added to the dish containing the wet silage sample before it is enclosed inside the bomb and has been found to give complete ignition of the sample. Because the energy content of the silage is found by difference, precision is lower than with dried samples, but this is fully justified because of the inevitable losses of volatiles occurring when silage is oven-dried.

Corrections

A number of corrections may be necessary in precise calorimetric determinations:

Thermometer calibration. This results from slight variations in the bore of the thermometer stem, which are corrected from a calibration certificate supplied with the thermometer. The present thermometers have been N.P.L. calibrated to $\pm 0.002^\circ\text{C}$ at 0.5°C intervals. Assuming linear variation between calibration points it has been shown (Barker, J. E. *et al.*, 1955) that the error in *corrected* temperature reading with such a thermometer can be $\pm 0.004^\circ\text{C}$, so that the error in corrected temperature *rise* can be $\pm 0.008^\circ\text{C}$. As the temperature rise is generally about 2°C , this error can account for a large part of the coefficient of variation of energy determinations noted above. It is evident that any worthwhile reduction of this C.V. will require the use of much more precise thermometers.

Emergent stem correction. Calorimeter thermometers are calibrated under conditions of total immersion, but are used with the 'emergent stem' in air, which may be as much as 5°C cooler than the water surrounding the thermometer bulb. It has, however, been calculated that the maximum error from this source leads to an underestimate of the temperature rise of the bomb by 0.002°C , which is negligible compared with the calibration errors of the present thermometers.

Ignition errors. These arise from the heat generated when the ignition wire fuses. However, if this is regarded as a constant heat release (q) the error in determination (e) is arrived at as follows:

$$e = q \frac{H}{Q} - q$$

where H = heat release from the sample and Q = heat release from benzoic acid used for calibration. As long as H and Q are not markedly different it has been shown (*ibid*) that this error is negligible.

Acid corrections. It has been found that combustion of some of the nitrogen and sulphur in samples of herbage and faeces to nitric and

sulphuric acids releases less than 0.2 and 0.3 calories, respectively, per 100 calories of heat release from the sample. The amounts of these acids produced can be measured by estimating the total acids by titration with alkali, and the SO_4 by precipitation with BaCl_2 . If this correction is not made, the maximum error in energy content is 0.5%. Corrections for acid production need only be made in the most precise energy-balance studies, as errors in the measurement of the energy contents of herbage intake, and of faecal and urinary excretion, will tend to cancel each other.

CHAPTER 24

COLD-STORAGE FACILITIES

At the Institute a large cold-store measuring $52 \times 20 \times 10$ ft (Fig. 17), which has been in operation for 5 years, has introduced a remarkable degree of flexibility into the investigations being carried out. Cold-storage at 5°F was introduced as a method for providing herbage of constant composition for digestibility experiments (see p. 86) but its use was soon adopted for the following additional purposes:

(1) The majority of herbage samples taken during summer for botanical analysis are kept in cold-storage and analysed during winter, when pressure of work is reduced (p. 45).

(2) Soil-core and other soil-samples are held in cold-storage until they can be further processed (p. 105). Freezing has the further advantage of rendering certain soils more easily separable from roots.

(3) Total faeces and urine collections are kept in cold-storage over the period of a digestibility experiment, and weighed and sampled at the end of the experiment (p. 90). This markedly reduces the labour involved in these experiments.

(4) Samples of fresh faeces from all digestibility experiments, of adequate size for possible future chemical analyses, can be stored indefinitely.

(5) Undried herbage samples for certain chemical analyses can be stored until required. The suitability of cold-storage for any particular analysis must of course be considered: thus it is likely that changes in certain labile components, e.g. soluble sugars, can occur even during storage at 5°F .

(6) It frequently happens that more herbage samples are brought in from the field than can reasonably be weighed and sub-sampled for dry-matter determinations on that day. These samples can be kept overnight in the cold-store and weighed and sampled on the following day.

(7) In this integrated cold-storage unit, facilities for storage of sample joints from animal slaughter experiments (p. 77), and of materials from parasitological experiments (p. 83) are also available.

General points

While the cold-storage facilities installed at the Institute are unlikely to be exactly those required at other centres, the following general points are worth recording:

(a) For safe storage of large amounts of herbage, a cold-store temperature as low as 5°F has been found necessary.

(b) Where large amounts (e.g. >500 lb) of herbage are to be frozen at one time, facilities for quick-freezing are desirable. These can be provided in a specialized quick-freeze chamber (Fig. 17) or by the

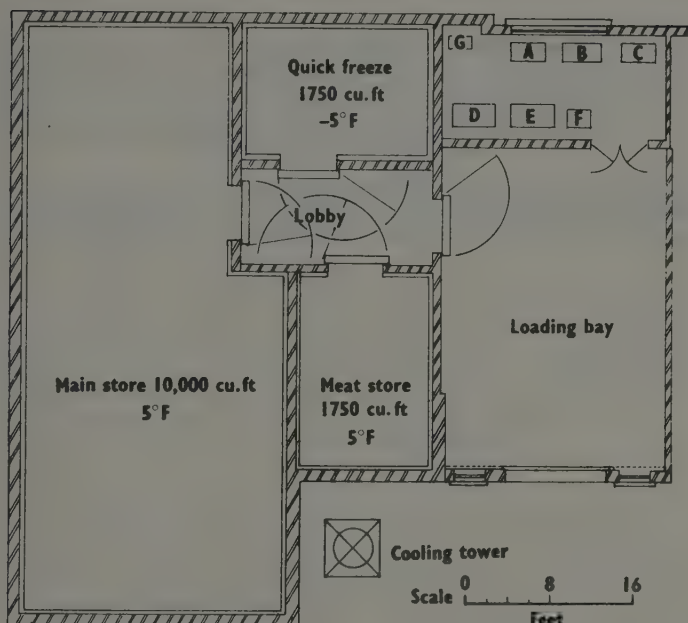


FIG. 17. Detail of cold-store installed at the Grassland Research Institute. A, B & C— $2\frac{1}{2} \times 2\frac{1}{2}$ in. quad water-cooled Hallmark compressor and condenser units. A & B—for quick-freeze, C—for Meat Room. D & E—Two $4 \times 3\frac{1}{2}$ in. twin compressor and condenser units for Main Store. F—Control panel. G—Condenser pumps. Quick-freeze and Meat-Room cooling by air blast. Insulation: 6-in. cork throughout.

use of fans to give rapid air-circulation in a main cold-store. These fans would only operate while herbage is being frozen. In this system the refrigeration equipment installed must have a larger capacity than would be required merely for maintaining low temperature in the store.

(c) When large samples of herbage are to be frozen, it is essential that cold air should circulate freely round each sub-unit (e.g. sack) of herbage to allow rapid freezing.

(d) Herbage in cold-storage tends to lose moisture, which condenses as ice on the cooling pipes in the store ('freeze-drying' at atmospheric pressure). Such drying of herbage is often undesirable, and icing-up of the cooling pipes reduces the efficiency of refrigeration. Drying should be avoided by enclosing herbage samples in polythene bags where possible, and by close stacking of sacks of herbage to be used for feeding.

(e) Cooling of the main cold room in the present store is by a network of refrigeration pipes located on the walls and ceiling of the store. When

these are defrosted, ice falls on to the stored materials, and this is undesirable. It would seem preferable to cool such a store by slow circulation of the air from the store over a bank of cooling coils located in a separate compartment. When these coils ice-up they can be defrosted without interfering with the stored materials. When quick-freezing is required the rate of air-circulation can be increased by speeding up the circulating fans.

(f) As noted on p. 89, when a large quantity of herbage is stacked, the base of the stack should be raised from the floor of the cold-store so as to allow air to circulate under the stack and reduce the risk of heating.

CHAPTER 25

DETERMINATION OF THE CHROMIC-OXIDE CONTENT OF FAECES

Three methods for determining the chromic-oxide content of faeces' samples from herbage intake experiments have been tested.

Fusion method (Schurch et al., 1950)

The milled faeces sample is ashed and the ash fused with sodium hydroxide alone at 900°C, or with sodium hydroxide and an oxidizing agent (sodium peroxide or potassium nitrate) at 600°C. After cooling, the chromium present is oxidized to the hexavalent state and estimated as dichromate. While complete recovery of chromium is possible with careful manipulation, the method is lengthy and the nickel crucibles for fusion last for only a small number of determinations.

Oxidation with perchloric acid (Bolin et al., 1952)

The chromium in the sample is oxidized to the hexavalent state by heating the faeces (either the milled sample or the ashed sample) with perchloric acid. The dichromate is then generally estimated colorimetrically. Unless special precautions are taken, some chromium can be lost as volatile chromyl chloride and reduction can also occur after the oxidation stage, in each case leading to low recovery of chromium. Thus this method does not seem suitable for routine use.

Solution in phosphoric acid and oxidation with bromate

This method was described by Christian & Coup (1954) and has been adopted with minor modifications to allow samples with a wide range of ash- and chromic-oxide contents to be analysed. The method is rapid and recovery of chromic oxide is within 2% of the theoretical figure.

Method. 4–5 g of dried milled faeces are ashed overnight at 600°C in a nickel crucible. The ash is brushed into a 250-ml conical flask, and the crucible washed with 4 ml of water. 5 ml of phosphoric acid/manganese sulphate solution and 3 ml potassium bromate solution (see below) are added and the mixture immediately digested on a hot plate until effervescence ceases and a purple colour appears (7–15 min). After cooling for 15–30 sec, 10 ml sulphuric acid/manganese sulphate solution and a further 4 ml potassium bromate solution are added. Any solid material adhering to the flask is dispersed with a glass rod and the solution boiled for 5 min until it turns orange-red from free bromine (plenty of silica chips are added to avoid 'bumping'). 100 ml water and 5 ml clearing solution are added and the mixture boiled until starch-iodide paper shows it to be free from bromine (c. 10 min). It is then cooled and titrated with ferrous ammonium sulphate, using ferroin as an indicator.

Solutions. (1) Potassium bromate. 4.5% w/v solution. (2) Phosphoric acid/manganese sulphate. 30 ml 10% $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ in 1 l Analar orthophosphoric acid. (3) Sulphuric acid/manganese sulphate. 5 ml 10% $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ in 1 l 50% sulphuric acid. (4) Clearing mixture. 125 g ammonium sulphate and 70 ml Analar hydrochloric acid in 1 l of water. (5) N/20 ferrous ammonium sulphate. 100 g ferrous ammonium sulphate made up to 5 l with 5% sulphuric acid.

Standardization of ferrous ammonium sulphate. To 25 ml N/20 potassium dichromate add 100 ml water, 6 ml Analar orthophosphoric acid, 5 ml Analar sulphuric acid and 2 drops ferroin. Titrate with ferrous ammonium sulphate. 1 ml N/20 ferrous ammonium sulphate $\equiv$ 1.266 mg Cr_2O_3 .

CHAPTER 26

NOTES ON THE DETERMINATION OF TOTAL NITROGEN BY THE KJELDAHL METHOD

While no full description of the techniques of chemical analysis in use at the Institute is given in this bulletin, the following features of the method used for the determination of total nitrogen may be of interest: over 18,000 determinations are carried out annually by two technical assistants.

(1) Milled samples are weighed on to tared papers (pipe or cigarette papers, which have a negligible N-content, are very suitable). These are folded and twisted to enclose the sample and can be dropped down the neck of a Kjeldahl flask without the sample adhering to the neck, even when the latter is wet.

(2) After weighing, the twists are placed in numbered compartments in a small tray. This is carried direct to the Kjeldahl room, so avoiding the taking of large numbers of flasks into the balance room.

(3) The catalyst mixture is added in the form of tablets. These are produced commercially and a number of mixtures are available [30]. At this Institute tablets containing 4.75 g of potassium sulphate and 0.25 g of mercuric oxide are used, 2 tablets being used with 25 ml conc. H_2SO_4 for a 1-g sample (dry weight).

(4) After digestion and dilution, a tablet containing 2 g sodium thiosulphate [30] is added to precipitate the mercury before the distillation stage.

(5) The use of an automatic titrator has been found invaluable in a unit where up to 144 titrations may be carried out in one day.

(6) The present unit is all-electric and individual monel-metal sheathed elements (350 W for digestion, 500 W for distillation) [31] have been found excellent for this purpose. A 6-flask digestion unit is shown in Plate 28, and 6 of these units are housed in a fume-cupboard with a floor area measuring 7×4 ft.

(7) Flasks and splash-bulbs fitted with B19 standard sockets and cones have proved superior to rubber bung fittings, but their initial cost is higher.

(8) To minimize the dangers involved in handling large amounts of caustic soda, 40% caustic soda solution is purchased in 7-cwt drums. From these the solution is delivered directly through a pipe to the distillation bench, by applying a pressure of 2 lb/sq. in. to the liquid in the drum.

APPENDIX 1

CONTRIBUTORS TO THE BULLETIN

Introduction	WILLIAM DAVIES, D.Sc. (Wales), Hon. D.Sc. (N.Z.)
Part I	C. D. KEMP, B.Sc., Dip.Stat.
„ II	J. O. GREEN, M.Sc. D. D. KYDD, M.A. D. A. LAMBERT, B.Sc. R. D. WILLIAMS, B.Sc., Ph.D. T. E. WILLIAMS, B.Sc.
„ III	F. E. ALDER, D.F.C., B.Sc., M.A. T. H. BROWN, B.Sc. D. T. CHAMBERS, M.Sc. C. E. HARRIS R. V. LARGE, B.Sc. D. J. MINSON, B.Sc., Ph.D., A.R.I.C. W. F. RAYMOND, M.A., F.R.I.C. C. R. W. SPEDDING, M.Sc., Ph.D. J. C. TAYLER, M.A., Dip.Ag.
„ IV	C. R. CLEMENT, B.Sc., Ph.D. A. J. HEARD, B.Sc., M.Sc. W. STILES, M.Sc. T. E. WILLIAMS, B.Sc.
„ V	H. K. BAKER, B.Sc., Ph.D., Dip.Ag.
„ VI	M. L. BARNES R. J. CANAWAY, Assoc. Brit. I.R.E. C. E. HARRIS E. C. JONES, B.Sc., F.R.I.C. W. F. RAYMOND, M.A., F.R.I.C.

APPENDIX 2

LIST OF PROPRIETARY PRODUCTS REFERRED TO IN THE TEXT

This list contains only items of equipment that have been used at the Grassland Research Institute and found suitable for the purpose indicated in the text. It is not intended either to show all sources of supply of suitable equipment or to imply that items mentioned are necessarily the most suitable.

TEXT REF.	TYPE OF EQUIPMENT	MANUFACTURER	NOTES
<i>Herbage cutting</i>			
1	Hedge trimmer & generator	Tarpen Engineering Co., Coronation Road, Park Royal, London, N.W. 10.	Modified as noted on p. 39.
2	Motor scythe	John Allen & Sons, Ltd., Cowley, Oxford, England.	"Autoscythe", model T/S preferred to model F. The attachment for cut- ting kale was designed for model T/S.
3	Sheep-shearing head & motor	Wolseley Engineering Co., Electric Avenue, Witton, Birmingham 6.	"Ringer" shearing head, Series II, wide pattern with special grass- cutting comb. "Merrytiller" wheeled power unit. Plastic covered flexible driving shaft.
<i>Weighing equipment</i>			
<i>Animals</i>			
4	Chain hoist	George W. King Ltd., Argyle Works, Steven- age, Herts.	"Matom" hoist, capacity 200 lb, 3 phase.
5	Livestock weighbridge	Vandome & Hart Ltd., N. London Ironworks, Wenlock Road, London, N. 1.	Class S weighbridge, capacity 2800 lb.
6	Platform weighbridge	W. T. Avery Ltd., Avery House, Clerkenwell Garden, London, E.C. 1.	
7	Weighbridge & crush	Gascoigne Ltd., Berkeley Avenue, Reading, Berks.	Gascoigne "Weigh-Crush".

APPENDIX 2 (*continued*)

TEXT REF.	TYPE OF EQUIPMENT	MANUFACTURER	NOTES
	<i>Weighing equipment</i>		
	<i>Herbage</i>		
8	Automatic balance	August Sauter K.G., Ebingen, Württemberg, West Germany.	e.g. ASE fully automatic scale, Type SA 100, 2.5 kg × 1 g.
9	Dial balance	Berkel Auto Scale Co. Ltd., Minerva Road, Park Royal, London, N.W. 10.	Model 101S, 25 kg, 1 kg × 5 g.
10	Spring balance	Precision Engineering Co. Ltd., Meadow Road, Reading, Berks.	"Weighmaster" balance, 9-in. dial, 50 lb × 0.2 lb.
	<i>Animal experiments</i>		
11	Chromic oxide capsules	R. P. Scherer Ltd., Bath Road, Slough, Bucks.	Filled capsules containing 1 g or 10 g of Cr ₂ O ₃ .
12	Faecal collection bags	Avon India Rubber Co. Ltd., Melksham, Wilts.	Available for sheep and cattle.
13	Faeces mixer	Hobart Mfg. Co., Hobart Corner, New Southgate, London, N. 11.	Model ME800, capacity up to 80 lb wet faeces.
14	Lamb- & calf-feeders	Dalton Supplies Ltd., Nettlebed, Henley-on-Thames, Oxon.	"Lambar" and "Calfeteria".
15	Lead-free paint	Walter Carson & Sons Ltd., Grove Works, Lombard Road, London, S.W. 11.	CR Paint B025239/1 and /2.
16	Polystyrene particles	General Chemical and Pharmaceutical Co. Ltd., Judex Works, Sudbury, Wembley, Middlesex.	Available in green, red, yellow, blue or white in 1-, 2-, or 5-kg packs.
17	Sheep & cattle harness	Turf and Travel Ltd., Oak End Way, Gerrards Cross, Bucks.	
18	Water bowls	Fordham Pressings Ltd., Dudley Road, Wolverhampton.	Fordham twin portable drinker.
19	Whatman absorption blocks	Innes Walker (Eng.) Co. Ltd., 30/34 Stanley Street, Glasgow, S.1. H. Reeve Angel & Co. Ltd., 9, Bridewell Place, London, E.C.4.	"Clydebuilt" bowl.

Field Experiments

- 20 Combine harvester Massey Harris Ferguson Ltd., Fletchamstead Highway, Coventry.
- 21 Expanded ebonite Expanded Rubber Co. Ltd., 675 Mitcham Road, Croydon, Surrey.
- 22 Insulated boxes Expanded Rubber Co. Ltd., 675 Mitcham Road, Croydon, Surrey.
- 23 Post driver Swindon Machinery Co. Ltd., 38 Oxford Street, Daventry, Northants.
- 24 *Laboratory Equipment* Adiabatic bomb calorimeter Gallenkamp Towers Ltd., 17/29 Sun Street, London, E.C.2.
- 25 Blender M.S.E. Ltd., Manor Royal, Crawley, Sussex.
- 26 Drying oven Birmingham & Blackburn Construction Co. Ltd., Armoury Close, Bordesley Green, Birmingham.
- 27 Drying trays Broadbear Ltd., Providence Tin Works, Audley Street, Reading, Berks.
- 28 Dust extraction Dallow Lambert & Co. Ltd., Thurmaston, Leics.
- 29 Electrolytic meter Siemens Schuckert Ltd., Faraday Works, Great West Road, Brentford, Middlesex.
- 30 Kjeldahl tablets Thompson Capper Ltd., Speke Hall Road, Liverpool 24.
- 31 Laboratory heaters Backer Electric Co. Ltd., Fitzwilliam Road, Rotherham, Yorks.
- 32 Laboratory mill Christy & Norris Ltd., Chelmsford, Essex.
- 33 Macerator mill Établissements Bonnet, 9, Rue Grenette, Villefranche, Rhône, France.
- 34 Photometer Megatron Ltd., 115a Fonthill Road, London, N.4.

No. 726. 8-25-ft cut, self-propelled.
 "Onazote".
 Tompkin "Postmaster" No. 1.
 "Ato-Mix" with monel metal container.
 "Unitherm" oven (N.B. weight of oven >2000 lb).
 For sizes, see p. 137.
 "Dustmaster" series.
 Available in a range of weights and compositions.
 350W & 500W laboratory heaters, drg. C6787/2.
 C and N mills, size 5 in. or 8 in.
 Coupemel mill.

APPENDIX 2 (*continued*)

TEXT REF.	TYPE OF EQUIPMENT	MANUFACTURER	NOTES
	<i>Laboratory Equipment</i>		
35	Sample storage cartons	Mono Containers Ltd., Cumberland Avenue, London, N.W.10.	Specify plain screw-cap waxed con- tainers No. 80=6 oz., No. 240= 22 oz.
36	Solarimeter	P. J. Kipp & Zonen, Delft, The Netherlands.	

APPENDIX 3

CONVERSION TABLE

<i>Length</i>		
1 inch (in.)		=2.540 cm
1 foot (ft)	=12 in.	=30.48 cm
1 yard (yd)	=3 ft	=91.44 cm
<i>Area</i>		
1 square inch (sq.in.)		=6.452 cm ²
1 square foot (sq.ft)	=144 sq.in.	=929.0 cm ²
1 square yard (sq.yd)	=9 sq.ft	=0.8361 m ²
1 acre	=4840 sq.yd	=0.4047 ha
<i>Volume and Capacity</i>		
1 pint	(=1.201 U.S. fluid pints)	=0.5683 litre (l)
1 gallon (gal)	=8 pints	=4.546 l
1 cubic foot (cu.ft)		=28.32 l
<i>Weight</i>		
1 ounce (oz)		=28.35 g
1 pound (lb)	=16 oz	=453.6 g
1 hundredweight (cwt)	=112 lb	=50.80 kg
1 ton	=20 cwt	=1016.0 kg
<i>Temperature</i>		
32°F	=0°C	
212°F	=100°C	
<i>Combined Units</i>		
1 lb per acre	=1.121 kg per ha	
1 cwt per acre	=125.5 kg per ha	
1 lb per sq.in.	=70.31 g per cm ²	
1 lb. in.	=0.01152 kg.m	
1 foot candle (ft c)	=10.76 lumen per sq.m	

LITERATURE CITED

- AGRICULTURAL RESEARCH COUNCIL STUDY GROUP ON BEEF CARCASS QUALITY. 1959. Methods of beef carcass evaluation. *Rep. A.R.C. Beef Study Group* pp. 7, mimeo.
- ALDER, F. E. 1955. Grassland management for meat production. *J. Brit. Grassl. Soc.* 10, 115-25.
- ALDER, F. E. 1960. The use of twin beef cattle at the Grassland Research Institute, Hurley, England. *Proc. 7th study meet. Europ. Assoc. Anim. Prod.* 57-62.
- ALDER, F. E. & REDFORD, R. A. 1958. Further observations on grassland management for meat production. *J. Brit. Grassl. Soc.* 13, 239-46.
- AMERICAN SOCIETY OF AGRONOMY in joint committee with Amer. Dairy Sci. Assoc., Amer. Soc. Anim. Prod. & Amer. Soc. Range Mgmt. 1952. Pasture and range research techniques. *Agron. J.* 44, 39-50.
- ARMSTRONG, S. F. 1907. The botanical and chemical composition of the herbage of pastures and meadows. *J. agric. Sci.* 2, 283-304.
- ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1955. Official methods of analysis. 8th edn, pp. xvi+1008, bibl. at end of chaps, Washington: Ass. Off. Agric. Chem.
- BAILEY, P. H. *et al.* 1957. Differential loss of dry matter in the laboratory grinding of herbage samples. *J. Brit. Grassl. Soc.* 12, 157-65.
- BALCH, C. C. *et al.* 1951. Apparatus for the separate collection of faeces and urine from cows. *J. agric. Sci.* 41, 98-101.
- BARKER, A. S. *et al.* 1955. The assessment and recording of the utilized output of grassland. *J. Brit. Grassl. Soc.* 10, 67-79.
- BARKER, J. E. *et al.* 1955. Studies in bomb calorimetry. 4. Corrections. *Fuel, Lond.* 34, 303-16.
- BOLIN, D. W. *et al.* 1952. A simplified method for the determination of chromic oxide. *Science* 116, 634-5.
- BREMNER, J. M. 1955. Personal communication.
- BREMNER, J. M. & SHAW, K. 1955. The determination of ammonia and nitrate in soil. *J. agric. Sci.* 46, 320-8.
- BRODIE, B. B. *et al.* 1949. The estimation of antipyrine in biological materials. *J. biol. Chem.* 179, 25.
- BROWN, Dorothy. 1954. Methods of surveying and measuring vegetation. *Bull. 42 Commonw. Bur. Past. Fld Crops* pp. xv+223.
- CALLOW, E. H. 1947. Comparative studies of meat. 1. The chemical composition of fatty and muscular tissue in relation to growth and fattening. *J. agric. Sci.* 37, 113-29.
- CALLOW, E. H. 1948. Comparative studies of meat. 2. The changes in the carcass during growth and fattening and their relation to the chemical composition of the fatty and muscular tissues. *J. agric. Sci.* 38, 174-99.
- CALLOW, E. H. 1949. Comparative studies of meat. 3. Rates of fattening in relation to the percentage of muscular and fatty tissue in a carcass. *J. agric. Sci.* 39, 347-58.
- CALLOW, E. H. 1950. Comparative studies of meat. 4. Rates of fattening in relation to the deposition of fat and protein in the fatty and muscular tissues of meat carcasses. *J. agric. Sci.* 40, 1-8.
- CALLOW, E. H. 1955. Carcass wastage in cattle. *1st interim Rep. low Temp. Res. Sta., Cantab.* pp. 7, mimeo.
- CALLOW, E. H. 1957. Carcass wastage in cattle. *2nd interim Rep. low Temp. Res. Sta., Cantab.* pp. 6, mimeo.
- CALLOW, E. H. 1958. Personal communication.
- CANAWAY, R. J. *et al.* 1955. The automatic recording of animal behaviour in the field. *Electron. Engng* 27, 102-5.

- CHRISTIAN, K. R. & COUP, M. R. 1954. Measurement of feed intake by grazing cattle and sheep. 6. The determination of chromic oxide in faeces. *N.Z. J. Sci. Tech.* 36 (A), 328-30.
- CLARK, N. A. & OGG, C. L. 1942. A wet combustion method for determining total carbon in soils. *Soil Sci.* 53, 27-35.
- CLEMENT, C. R. & WILLIAMS, T. E. 1958. An examination of the method of aggregate analysis by wet sieving in relation to the influence of diverse leys on arable soils. *J. Soil Sci.* 9, 252-66.
- COCHRAN, W. G. & COX, G. M. 1957. Experimental designs. 2nd edn, pp. xiv+616, bibl. at end of chaps. New York: J. Wiley & Sons.
- COLOVOS, N. F. *et al.* 1957. Errors in drying silage and faeces for protein and energy determinations. Improved procedures. *J. Dairy Sci.* 40, 173-9.
- CORBETT, J. L. 1959. Paper as a carrier of chromium sesqui-oxide. *Nature, Lond.* 182, 1014-16.
- CORBETT, J. L. 1960. Faecal-index techniques for estimating herbage consumption by grazing animals. *Proc. 8th internat. Grassl. Congr.* 438-42.
- CORBETT, J. L. & GREENHALGH, J. F. D. 1960. Measurement of the quantities of herbage consumed by grazing animals. [Cont. in] Chemical aspects of the production and use of grass. *Monogr. 9 Soc. chem. Ind., Lond.* 167-180.
- COWLISHAW, S. 1951. The effect of sampling cages on the yields of herbage. *J. Brit. Grassl. Soc.* 6, 179-82.
- COX, C. P. *et al.* 1956. The direct evaluation of pasture in terms of milk production of individually grazed cows. *J. Brit. Grassl. Soc.* 11, 107-18.
- CRAMPTON, F. W. 1942. The design of animal husbandry experiments. *J. Anim. Sci.* 1, 263-76.
- CROFTON, H. D. 1954. The vertical migration of infective larvae of strongyloid nematodes. *J. Helminth.* 28, 35-52.
- CUNLIFFE, G. & CROFTON, H. D. 1953. Egg sizes and differential egg counts in relation to sheep nematodes. *Parasitology* 43, 275-86.
- DAVIES, A. W. *et al.* 1948. A new technique for the preparation and preservation of herbage samples. *J. Brit. Grassl. Soc.* 3, 153-8.
- DAVIES, William. 1936. The grasslands of Wales. [Cont. in] A survey of the agricultural and waste lands of Wales. By R. G. Stapledon [Ed.] 13-107, London: Faber & Faber.
- DAVIES, William. 1941. The grassland map of England and Wales explanatory notes. *Agriculture, Lond.* 48, 112-21.
- DICKMANS, G. & ANDREWS, J. S. 1933. A comparative morphological study of the infective larvae of the common nematodes parasitic in the alimentary tract of the sheep. *Trans. Amer. micr. Soc.* 52, 1-25.
- EDIN, H. 1926. [Prosecuted researches by indirect methods—based on the principle of a 'clue substance' ('marker substance')—for the determination of the digestibility of feed. The availability of chromium oxide as a 'clue substance', i.e., quantitative indicator.] *Medd. 309 CentAnst. Försöksv. Jordbr., Stockh.* pp. 80.
- FENTON, E. W. 1934. Grassland retrogression in Devonshire permanent pastures. *J. Ecol.* 22, 279-88.
- FINNEY, D. J. 1957. Stratification, balance and covariance. *Biometrics*, 13, 373-86.
- GARRIGUS, W. P. 1934. The forage consumption of grazing steers. *Proc. Amer. Soc. Anim. Prod.* 66-9.
- GASSER, J. K. R. 1958. Use of deep-freezing in the preservation and preparation of fresh soil samples. *Nature, Lond.* 181, 1334-5.
- GERRARD, F. 1951. Meat Technology. 2nd edn, pp. 309, bibl. 39, illus., London: Leonard Hill.
- HAMADA, M. K. O. 1950. A study of growth and conformation in early and late maturing breeds of sheep. *Thesis for Ph.D. Univ. Edinburgh.*

- HANCOCK, J. 1953. Grazing behaviour of cattle. *Anim. Breed. Abstr.* **21**, 1-13.
- HARMSSEN, G. W. & VAN SHREVEN, D. A. 1955. Mineralization of organic nitrogen in soils. *Advanc. Agron.* **7**, 299-398.
- HARRINGTON, G. & POMEROY, R. W. 1959. The yields of wholesale cuts from carcasses of Aberdeen Angus crosses fattened on grass and in yards. *J. agric. Sci.* **53**, 64-77.
- HESTER, J. B. 1948. Operation of an industrial service laboratory for analyzing soil and plant samples. [Cont. in] Diagnostic techniques for soils and crops. By H. B. Kitchen [Ed.] 117, Washington: The Amer. Potash Inst.
- HEWITT, E. J. 1952. Sand and water culture methods used in the study of plant nutrition. *Tech. Comm. 22 Commonw. Bur. Hort. Plant. Crops*, pp. x+241.
- HIBBS, J. W. & CONRAD, H. R. 1958. High roughage system for raising calves based on the early development of rumen function. 8. Effects of rumen inoculations and chlortetracycline on performance of calves fed high roughage pellets. *J. Dairy Sci.* **41**, 1230-47.
- HOPPER, M. J. *et al.* 1960. Attachment for a motor-scythe when cutting kale. *J. agric. Engng Res.* **5**, 342-3.
- HUGHES, G. P. 1951. The seasonal output of pasture sown with ultra-simple seeds mixtures. *J. agric. Sci.* **41**, 203-13.
- HUGHES, G. P. 1952. The comparative seasonal output of ultra-simple and general-purpose seeds mixtures. *J. agric. Sci.* **42**, 413-21.
- HUGHES, G. P. 1953. Beef behind the wire. *Fmrs' Wkly*, **39**, No. 23, 74.
- HUGHES, G. P. & REID, D. 1951. Studies on the behaviour of cattle and sheep in relation to the utilization of grass. *J. agric. Sci.* **41**, 350-66.
- JENKINS, H. V. 1959. An airflow planimeter for measuring the area of detached leaves. *Plant Physiol.* **34**, 532-6.
- JOHNSTONE-WALLACE, D. B. & KENNEDY, W. K. 1944. Grazing management practices and their relationships to the behaviour and grazing habits of cattle. *J. agric. Sci.* **34**, 190-7.
- JONES, L. I. 1937. The comparative value of pastures as measured by the grazing animal. *Rep. 4th internat. Grassl. Congr.* 386-94.
- JOUBERT, D. M. 1956. An analysis of factors influencing post-natal growth and development of muscle fibre. *J. agric. Sci.* **47**, 59-102.
- KANE, E. A. *et al.* 1952. Diurnal variation in the excretion of chromic oxide and lignin. *J. Nutr.* **47**, 263-73.
- KEMP, C. D. 1960. Methods of estimating the leaf area of grasses from linear measurements. *Ann. Bot., Lond. N.S.* **24**, 491-9.
- KEMPTHORNE, O. 1957. Arrangements of pots in greenhouse experiments. *Biometrics* **13**, 235-7.
- LABORATORY PRACTICE. 1956. Laboratory equipment test report No. 15. The Unitherm laboratory drier. *Lab. Prac.* **5**, 204-7.
- LANCASTER, R. J. 1949. The measurement of feed intake by grazing cattle and sheep. 1. A method of calculating the digestibility of pasture based on the nitrogen content of faeces derived from the pasture. *N.Z. J. Sci. Tech.* **31** (A), Pt 1, 31-8.
- LANGER, R. H. M. & CANAWAY, R. J. 1958. An automatic device for the control of day length. *J. hort. Sci.* **33**, 58-61.
- LINE, C. & HEAD, M. J. 1960. *See* MINSON, D. J. *et al.* 1960.
- LOCKHART, L. W. 1954. Sampling of fleeces for yield, staple length, and crimps per inch measurement. *Aust. J. agric. Res.* **5**, 555-67.
- MACLUSKY, D. S. 1955. The quantities of herbage eaten by grazing dairy cows. *Proc. Brit. Soc. Anim. Prod.* 1955 45-51.
- MCDONALD, P. & DEWAR, W. A. 1960. Determination of dry matter and volatiles in silage. *J. Sci. Fd Agric.* **10**, 566-70.
- MICHEL, J. F. 1954. A contribution to the aetiology of fog fever. *Vet. Rec.* **66**, 381-5.

- MICHEL, J. F. & PARFITT, J. W. 1955. A contribution to the epidemiology of parasitic bronchitis in calves. *Vet. Rec.* **67**, 229-35.
- MILTON, W. E. J. 1947. The yield, botanical and chemical composition of natural hill herbage under manuring, controlled grazing and hay conditions. 1. Yield and botanical section. *J. Ecol.* **35**, 65-89.
- MINSON, D. J. & BROWN, Susannah A. 1959. Herbage nitrogen as an index of herbage organic matter digestibility. *Exp. Progr. II Rep. Grassl. Res. Inst., Hurley 1957-8* 99-103.
- MINSON, D. J. *et al.* 1960. A method for identifying the faeces produced by individual cattle or groups of cattle grazing together. *J. Brit. Grassl. Soc.* **15**, 86-8.
- MYERS, E. A. 1952. A field plot irrigator. *Progr. Rep. 83 Pa State Coll. Agric.* pp. 4.
- NEENAN, M. *et al.* 1959. The output of Irish pastures. *J. Brit. Grassl. Soc.* **14**, 78-87.
- OLBRYCHT, T. M. 1945. A new instrument for measuring animals. Standardization of measurements in cattle. *Emp. J. exp. Agric.* **13**, 199-202.
- OWEN, J. B. 1957. A study of the lactation and growth of hill sheep in their native environment and under lowland conditions. *J. agric. Sci.* **48**, 387-412.
- PAECH, K. & TRACEY, M. V. 1955-6. Modern methods of plant analysis. Vols 1-4, pp. 542, 626, 761, 766, bibl. at end of chaps, Berlin: Springer-Verlag.
- PÁLSSON, H. 1939. Meat qualities in the sheep with special reference to Scottish breeds and crosses. *J. agric. Sci.* **29**, 454-626.
- PARFITT, J. W. 1955. Two techniques used for the detection and enumeration of the larvae of *Dictyocaulus viviparus* in faeces and herbage. *Lab. Prac.* **4**, 15-16.
- PARFITT, J. W. 1958. A technique for the enumeration of helminth eggs and protozoan cysts in faeces from farm animals in Britain. *Lab. Prac.* **7**, 353-5.
- PEARCE, S. C. 1953. Field experimentation with fruit trees and other perennial crops. *Tech. Comm. 23 Commonw. Bur. Hort. Plant. Crops*, pp. x+131.
- PIGDEN, W. J. & BRISSON, G. J. 1956. Effect of frequency of administration of chromic oxide on its faecal excretion pattern. *Canad. J. agric. Sci.* **36**, 146-55.
- PIPER, C. S. 1950. Soil and plant analysis, pp. 368, Adelaide: Univ. of Adelaide.
- POMEROY, R. W. 1956. Consumer preference for meat. *Bull. 14 Inst. Meat* 3-9.
- PRESTON, T. R. 1957a. Milk substitutes and early weaning of calves. *Agriculture, Lond.* **64**, 429-33.
- PRESTON, T. R. 1957b. Artificial rearing of calves at pasture. *J. Brit. Grassl. Soc.* **12**, 178-86.
- RAYMOND, W. F. & HARRIS, C. E. 1954. The laboratory drying of herbage and faeces. *J. Brit. Grassl. Soc.* **9**, 119-30.
- RAYMOND, W. F. & MINSON, D. J. 1955. The use of chromic oxide for estimating the faecal production of grazing animals. *J. Brit. Grassl. Soc.* **10**, 282-96.
- RAYMOND, W. F. *et al.* 1953. Technique of measurement of digestibility of herbage. *J. Brit. Grassl. Soc.* **8**, 301-14.
- RAYMOND, W. F. *et al.* 1954a. Studies in the digestibility of herbage. 4. The use of faecal collection and chemical analysis in pasture studies. *b. Faecal index methods.* *J. Brit. Grassl. Soc.* **9**, 69-82.
- RAYMOND, W. F. *et al.* 1954b. The variation with age, of the ability of sheep to digest herbage. *J. Brit. Grassl. Soc.* **9**, 209-20.
- RAYMOND, W. F. *et al.* 1956a. The effect of management on herbage consumption and selective grazing. *Proc. 7th internat. Grassl. Congr.* 123-33.
- RAYMOND, W. F. *et al.* 1956b. Normal-acid fibre: a proposed analysis for the evaluation of roughages. 2. *Agric. Progr.* **30**, 120-4.
- RAYMOND, W. F. *et al.* 1957. An automatic adiabatic bomb calorimeter. *J. sci. Instrum.* **34**, 501-3.
- RAYMOND, W. F. *et al.* 1959. Further evidence on the effect of level of intake on the digestive efficiency of sheep. *J. Brit. Grassl. Soc.* **14**, 75-7.

- REID, J. T. *et al.* 1952. A procedure for measuring the digestibility of pasture forage under grazing conditions. *J. Nutr.* **46**, 255-69.
- SCHOLLENBERGER, C. J. 1945. Determination of soil organic matter. *Soil Sci.* **59**, 53-6.
- SCHURCH, A. F. *et al.* 1950. The use of chromic oxide as an index for determining the digestibility of a diet. *J. Nutr.* **41**, 629-36.
- SEARS, P. D. 1951. The technique of pasture measurement. *N.Z. J. Sci. Tech.* **33** (A), Pt 1, 1-29.
- SNEDECOR, G. W. 1946. Statistical methods. 4th edn., pp. xvi+485, bibl. at end of chaps, Ames: Iowa State College Press.
- SPEDDING, C. R. W. 1952. Variation in the egg content of sheep faeces within one day. *J. Helminth.* **26**, 71-86.
- SPEDDING, C. R. W. 1953. Variation in the nematode egg content of sheep faeces from day to day. *J. Helminth.* **27**, 9-16.
- SPEDDING, C. R. W. 1954. Effect of a sub-clinical worm-burden on the digestive efficiency of sheep. *J. comp. Path.* **64**, 5-14.
- SPEDDING, C. R. W. & LARGE, R. V. 1957. A point quadrat method for the description of pasture in terms of height and density. *J. Brit. Grassl. Soc.* **12**, 229-34.
- STAPLEDON, R. G. 1925. Permanent grass. *Farm Crops* **3**, 74-136, London: Gresham Publ. Co.
- STAPLEDON, R. G. *et al.* 1945. Vegetation: grasslands of England and Wales (1940) [Map.] Southampton: Director General, Ordnance Survey.
- STAPLEDON, R. G. *et al.* 1952. Explanatory text No. 5 vegetation: the grasslands of England and Wales [Map]. pp. 10, Chessington: Director General, Ordnance Survey.
- TAYLER, J. C. & LARGE, R. V. 1955. The comparative output of two seeds mixtures. *J. Brit. Grassl. Soc.* **10**, 341-51.
- TAYLOR, E. L. 1939. Technique for the estimation of pasture infestation by strongyloid larvae. *Parasitology* **31**, 473.
- THOMSON, W. & THOMSON, A. M. 1953. Effect of diet on milk yield of the ewe and growth of her lamb. *Brit. J. Nutr.* **7**, 263-74.
- TRIBE, D. E. 1950. The behaviour of the grazing animal: a critical review of present knowledge. *J. Brit. Grassl. Soc.* **5**, 209-24.
- TRICKETT, E. S. & MOULSLEY, C. J. 1956. An integrating photometer. *J. agric. Engng Res.* **1**, 1-11.
- TRICKETT, E. S. *et al.* 1957. Measurement of solar and artificial radiation with particular reference to agriculture and horticulture. *J. agric. Engng Res.* **2**, 86-110.
- WALKLEY, A. & BLACK, I. A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **37**, 29-38.
- WALLACE, L. R. 1948. The growth of lambs before and after birth in relation to the level of nutrition. *J. agric. Sci.* **38**, 93-153.
- WARREN WILSON, J. 1959. Analysis of the distribution of foliage area in grassland. [Cont. in] The measurement of grassland productivity. By J. D. Ivins [Ed.] 51-61, London: Butterworths.
- WATSON, S. J. 1948. Animals as means of evaluating pasture production. *Proc. Brit. Soc. Anim. Prod.* 9th meet. 7-43.
- WESEMAEL, J. Ch. VAN & LEHR, J. J. 1956. The application of the dichromate method for the determination of soil organic matter. *Proc. 6th internat. Congr. Soil Sci.* 593-9.
- WILLIAMS, R. D. 1959. A method of growing grass plants with separation of seminal and adventitious roots. *Nature, Lond.* **183**, 415.
- WILLIAMS, T. E. & BAKER, H. K. 1957. Studies in the root development of herbage plants. 1. Techniques of herbage root investigations. *J. Brit. Grassl. Soc.* **12**, 49-55.
- YATES, F. 1937. The design and analysis of factorial experiments. *Tech. Comm.* **35 Imp. Bur. Soil Sci.** pp. 95.

GLOSSARY

Baermann technique. This is based on the use of a glass funnel, with clamped rubber tubing attached, for the sedimentation of small particles from aqueous suspension.

B.S.F. British Standard fine.

Cannon. *Metacarpus* (foreleg) or *metatarsus* (hind leg).

Cavings. Short straw and debris from the threshing machine.

Crutch-clip. The operation of removing the wool from the tail and quarters.

Dressing-out percentage. $\frac{\text{Cold carcass weight}}{\text{Live weight}} \times 100$. Empty weight is used instead of live weight for greater accuracy where facilities are available.

Empty weight. The weight of an animal excluding the contents of the alimentary tract.

'Finish'. The level of fatness, judged in the live animal by eye and palpation, in the carcass by eye or by measurement.

Half-sibs. Animals of either sex having the same sire (or dam).

Hoggett. A ewe, ram or castrated male sheep older than 9 months, before shearing.

Hooks. *Tuber coxae*.

'In', 'out' cuts. The herbage on offer, and the residual herbage after grazing.

Ley: Artificially established, temporary grassland: a mixed crop of gramineous, leguminous and, occasionally, other herbaceous forage plants, or a simple crop of one such plant (e.g. red clover, lucerne, or Italian ryegrass), growing for a more or less pre-defined period in rotation with other crops. The duration of the ley may be one year or several years.

London and Home Counties style. One of a number of regional systems of cutting the beef carcass into wholesale joints; described by Gerrard (1951).

McMaster slide. A microscope slide with 3 sunken chambers (1.5 mm deep) used for helminth-egg counting.

P.C.D. Pitch circle diameter.

Pins. *Tuber ischii*.

Race. The space or access corridor between adjacent plots.

Sampling unit. One of a group of small areas, normally sited at random, which together provide a sample of herbage representative of a plot or field.

Sampling area. The composite area of the sampling units constituting a sample.

Sampling fraction. The sampling area expressed as a fraction of the area represented by the sample.

Store animals. Animals fed at a nutritional level which allows only slow growth.

Store periods. Periods during which animals are kept at a relatively low nutritional level.

Strip grazing. A system in which electric fencing is used to give the animals a fresh strip of herbage daily.

S.W.G. Standard wire gauge.

Thurls. Lateral surface of *trochanter major* of *femur*.

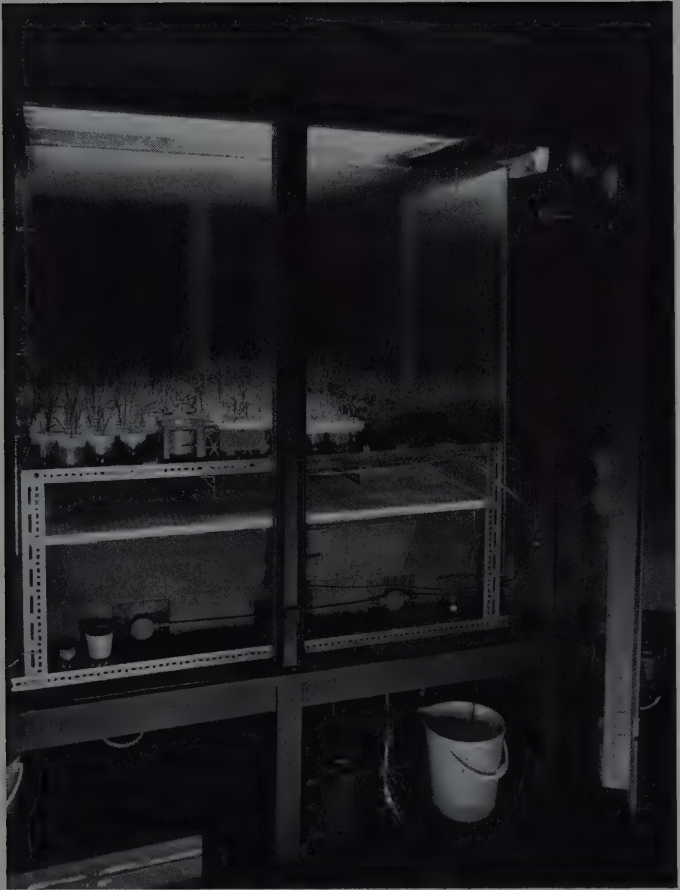


PLATE 1. Environmental chamber.



PLATE 2. Large pots for growth studies under sward conditions.



PLATE 3. Labelling of tillers with label and thread.



PLATE 4. Labelling of tillers with pigeon rings.



PLATE 5. Wire-netting fence for sheep showing movable iron posts.



PLATE 6. A trailer for weighing sheep of all ages.

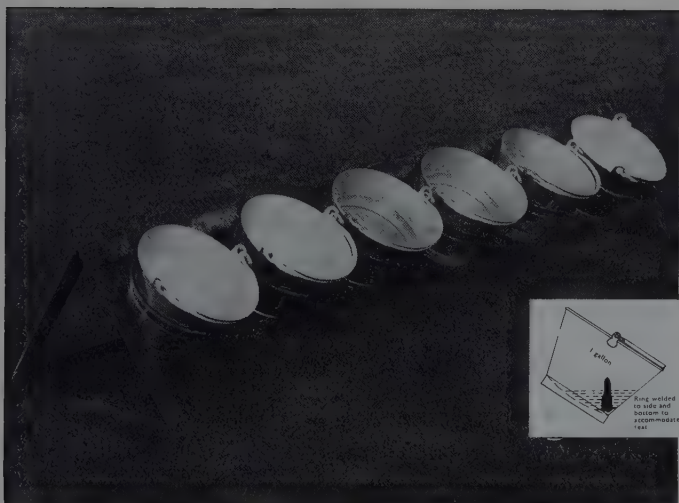


PLATE 7. Rack of buckets for artificial rearing of lambs. Inset showing position of teat in bucket.



PLATE 8. Group feeding of lambs.



PLATE 9. Sheep cages for digestibility experiments. The overhead railway and electric hoist for sheep-weighing can be seen.



PLATE 10. Sheep cage for digestibility experiments.

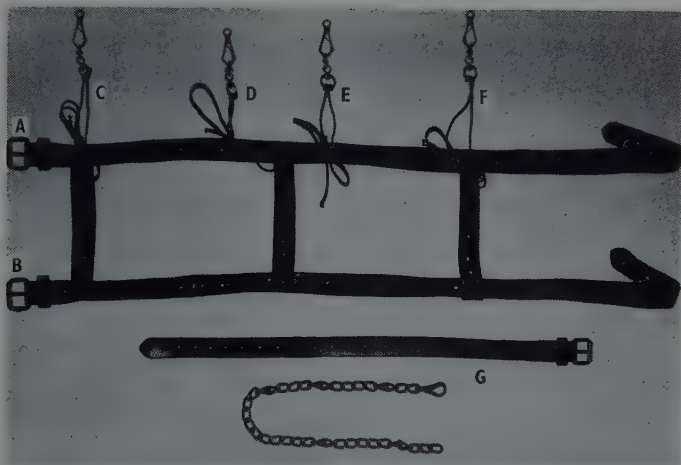


PLATE 11. Detail of sheep harness. A, rear girth strap (1 in. wide), B, fore girth strap, C, D, E, F, adjustable cords with scissor clips for holding faeces bags, G, leather collar and chain for securing sheep in cage.

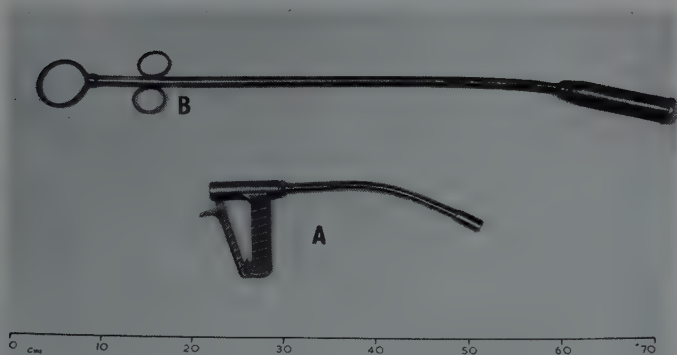


PLATE 12. Balling guns used for dosing chromic-oxide capsules. A, sheep, B, bullocks. In B the capsule is held in the 1-in. int. brass mouthpiece, and ejected by pushing in a rod attached to the large ring at the far end of the gun.



PLATE 13. View from offside of combine-harvester showing metal box (with sliding bottom) collecting grain from main auger; grain dropping into metal tray.



PLATE 14. Bagging the grain from the metal tray.



PLATE 15. Combine-harvester with canvas sheet hitched to it collecting straw, cavings and chaff.



PLATE 16. Weighing straw from a plot. The spring balance is suspended from a tractor-mounted hydraulically operated fore-loader. A new sheet is being hitched on to the harvester.



PLATE 17. An apparatus for small-plot irrigation.



PLATE 18. Detail of carriage and spray pipe.

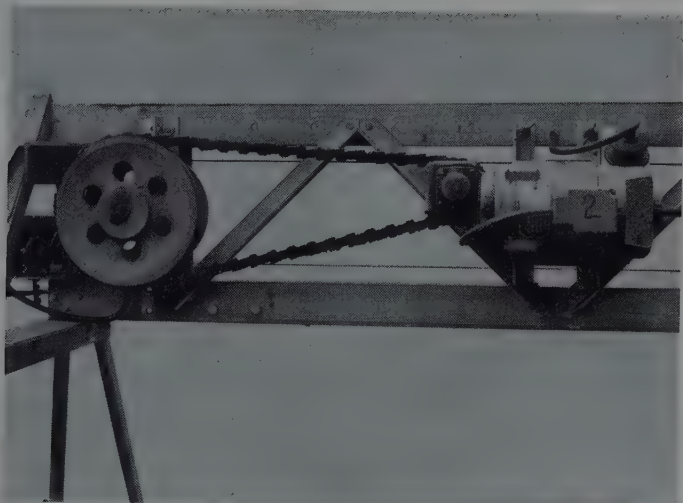


PLATE 19. The detachable electric motor for driving the winding drum is fitted to the bridge girder by wing nuts.



PLATE 20. The winding drum and reversing mechanism (switch cover removed).



PLATE 21. Mechanical post driver for taking soil cores for estimation of weight of roots; a spare sampler, striking head and extracted soil cores in the foreground.

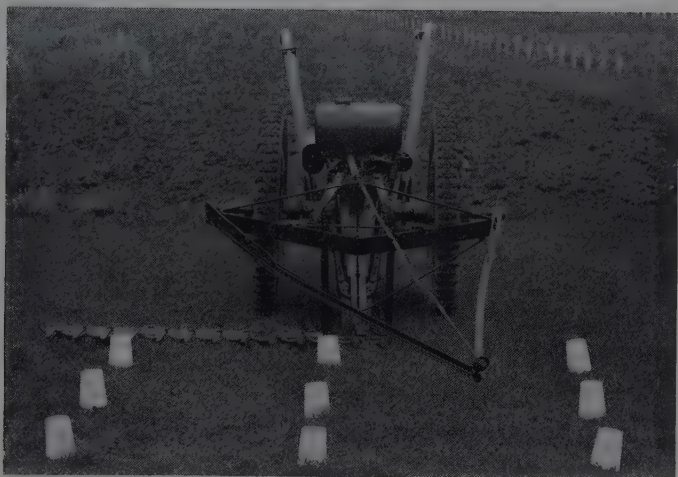


PLATE 22. Motor-scythe fitted with an attachment for cutting two rows of kale drilled two feet apart.



PLATE 23. Mobile unit used for herbage and botanical sampling.



PLATE 24. Trailer for plot cages.



PLATE 25. Trailer loaded with plot cages.



PLATE 26. Portable weighbridge and trailer.



PLATE 27. Portable weighbridge and trailer.

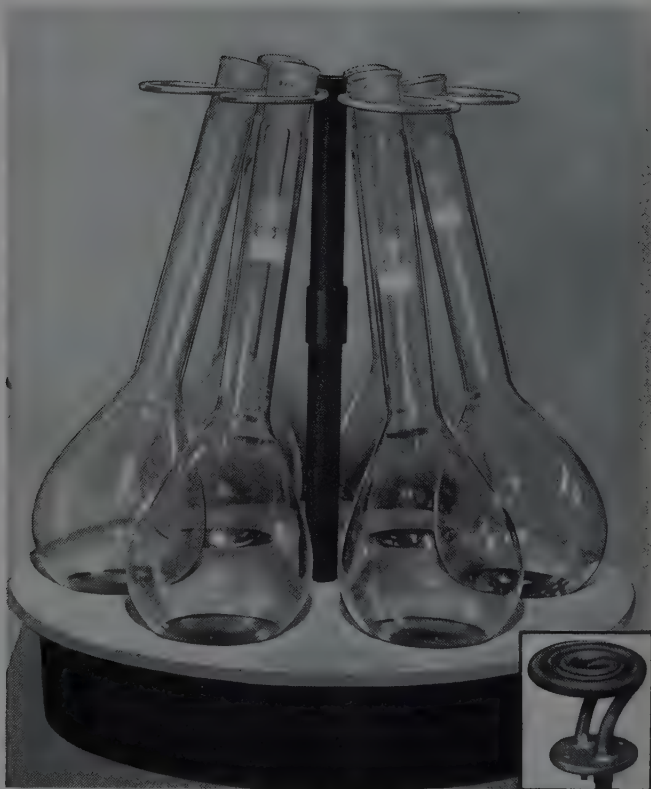


PLATE 28. Six-unit Kjeldahl digestion rack. Heating is by 350-watt totally enclosed elements [31] (see inset) or by low-voltage bars. The flask assembly rotates on the centre spindle to allow each flask to be handled easily.

PUBLICATIONS
of the
COMMONWEALTH BUREAU OF
PASTURES AND FIELD CROPS

Herbage Abstracts. A quarterly abstract journal on grasslands, fodder crops and their management. First issued 1931.

Annual subscription

Volume 31 (1961) 50s.

Volume 32 (1962) 70s.

Field Crop Abstracts. A quarterly abstract journal on the agricultural aspects of annual field crops. First issued 1948.

Annual subscription

Volume 14 (1961) 50s.

Volume 15 (1962) 70s.

Please write for a free specimen copy

Bulletins

NO.

44. The underground organs of herbage grasses. A. Troughton. Crown 4to. Cloth boards. 163 pp. (1957) 25s. 0d.

43. The storage of seeds for maintenance of viability. Dr. E. B. Owen. Crown 4to. Cloth boards. 81 pp. (1956) 20s. 0d.

42. Methods of surveying and measuring vegetation. Miss D. Brown. Crown 4to. Cloth boards. 223 pp., 45 illustrations. (1954) 35s. 0d.

Mimeographed Publications

1/1961. A bibliography of subterranean clover together with a descriptive introduction. D. E. Symon. Crown 4to. Paper cover. 122 pp. (1961) 12s. 0d.

3/1959. Bracken: a review of the literature. K. W. Braid. Crown 4to. Paper cover. 69 pp. (1959) 7s. 6d.

2/1959. Grazing control: laws and regulations in various countries for the control of grazing to prevent injury to grasslands. A. G. G. Hill. Crown 4to. Paper cover. 27 pp. (1959) 7s. 6d.

1/1959. Minor elements and their effects on the growth and chemical composition of herbage plants. R. Dorrington Williams. Crown 4to. Paper cover. 68 pp. (1959) 7s. 6d.

Obtainable through any major bookseller or direct from:

Commonwealth Agricultural Bureaux,
Central Sales,
Farnham Royal,
Bucks., England.

